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RECENT ADVANCES IN THE STUDY OF PLANT VIRUSES

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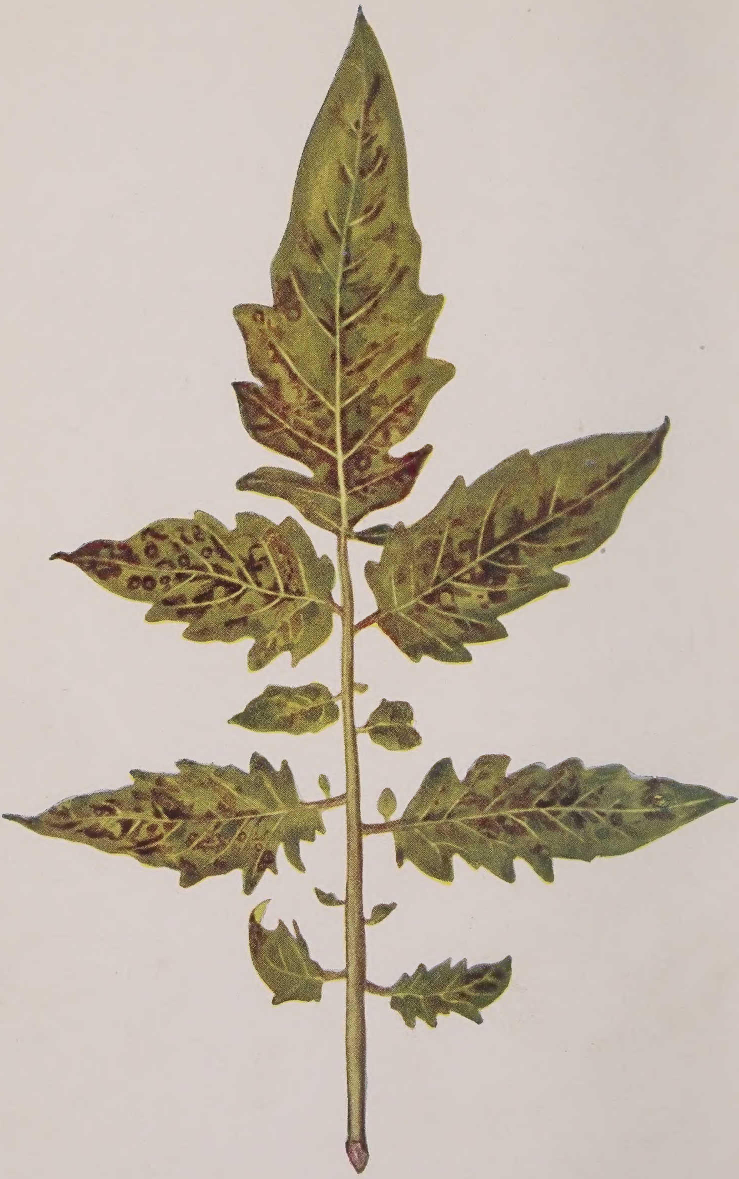

Spotted Wilt Disease of the Tomato, showing the characteristic bronze-coloured markings on the leaves.

RECENT ADVANCES
IN THE STUDY OF
PLANT VIRUSES

By
anley
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With Foreword by
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With 1 Coloured Plate and
67 Text-figures

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To
DAVID KEILIN
QUICK PROFESSOR OF BIOLOGY
IN THE UNIVERSITY OF CAMBRIDGE
THIS BOOK IS GRATEFULLY DEDICATED

ἡ γὰρ φύσις μεταβαίνει συνεχῶς ἀπὸ τῶν ἀψύχων εἰς τὰ ζῷα διὰ τῶν ζώντων μὲν οὐκ ὄντων δὲ ζῴων, οὕτως ὥστε δοκεῖν πάμπαν μικρὸν διαφέρειν θατέρον θάτερον τῷ σύνεγγυς ἀλλήλοις.

"Nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful."

Aristotle, *de partibus animalium*, IV., 5, 681a, 12.
(Translation *in* Rivers (28).)

FOREWORD

In recent years one of the most active branches of Plant Pathology has been the study of the virus diseases of plants, and an enormous literature has already accumulated on this subject, much of which is difficult of access. No comprehensive account of these investigations has hitherto appeared. The time is particularly opportune, therefore, for a critical survey of the present state of knowledge of this group of plant diseases, and it is especially appropriate that this survey has been made by Dr. Kenneth Smith, who is one of the most active and successful investigators in this field.

The value of this book has been enhanced by the comparisons made in it between plant and animal virus diseases, by the full account of the technique of investigating plant viruses and the diseases they cause, and by the indications given of profitable lines of investigation in the future.

Many of the problems concerning viruses may perhaps pertain to the border-line kingdom between the living and the non-living, and in this respect knowledge of these remarkable agents of disease is of special concern to all biologists.

Our information about plant viruses is still in a very tentative state in certain respects, and the author rightly points out that his book has not been written from the standpoint of providing a text-book. "Recent Advances in the Study of Plant Viruses," however, will prove to be a book of great interest to all workers on virus diseases and a most valuable guide to plant pathologists in general and others concerned with the healthy maintenance of cultivated plants. Above all, this book, I venture to think, will be a fertile stimulus to further investigation of this intriguing group of plant diseases.

F. T. BROOKS.

Botany School,
Cambridge.

PREFACE

THE study of the plant viruses is of comparatively recent development, and at the time of writing there exists no book upon the subject. The knowledge of the filterable viruses is at the moment in such a state of flux that in the writer's opinion the time is not ripe for a text-book. Nevertheless, the great and increasing importance of the plant viruses and their many affinities with the similar agents affecting animals make it desirable that there should be some kind of reference book available for the student and the plant virus worker. The present volume has therefore been written in the hope that it will serve the dual purpose, first of giving a fairly comprehensive survey of the present position of the knowledge concerning these interesting agents, and secondly of acting as a students' reference book until the progress and correlation of knowledge allow a more comprehensive treatise to be written. For the latter reason, certain subjects have been dealt with in greater detail than is usual in a book of this type.

In order to widen the sphere of usefulness of the book and to add to its interest, an endeavour has been made to draw comparisons in the behaviour of the plant and animal viruses respectively, so far as the limited knowledge of the writer on the latter subject would allow. It seems a pity that there should not be more collaboration or exchange of knowledge between the plant and animal virus workers when it is considered how closely in many ways the two types of viruses resemble each other.

As regards the terminology of the subject of the filterable viruses, the use of certain words has not been altogether consistent. As Rivers has pointed out, the stem of the verb *filter* is *filtr-*. Thus the adjective derived from it would ordinarily be *filtrable ;* nevertheless, the use of *filterable* has been adhered to in preference to *filtrable*, in view of the wide usage of the former word. The word *virosis*, coined in America to designate a " virus disease," has also been avoided, as being unnecessary and liable to be confused with "*viruses*." There is at present no word in general use descriptive of the science which involves the study of viruses, and here again the writer has not ventured to coin and use the obvious word "virology."

Grateful acknowledgment is due to Mr. J. P. Doncaster for making the drawings illustrating Figs. 9 and 10 and for his help in preparing the manuscript for press; to Dr. J. Henderson Smith for kindly reading and criticizing part of the manuscript; to Dr. W. J. Elford for his constructive criticisms of Chapter IV.; to the editor of *Biological Reviews* for his kind permission to use certain material from that Journal; to Mr. F. C. Bawden and Dr. H. H. Storey, who allowed the use of some of their unpublished work; and to Mr. E. R. Speyer, who supplied the drawing for Fig. 15 and some unpublished data on the specific characters of *Thrips tabaci*.

Various points have also been discussed with Mr. F. T. Brooks, F.R.S.; Prof. D. Keilin, F.R.S., and Dr. R. N. Salaman.

The writer is much indebted to the editor of the *Bulletin of Entomological Research* for the loan of the blocks of Figs. 12, 13 and 53; to the editor of *Discovery* for those of Figs. 4, 6, and 7; to the editor of the *Gardener's Chronicle* for that of Fig. 54; and to Prof. Paul Murphy and the Royal Dublin Society for the blocks of Figs. 51, 52. The coloured frontispiece and Figs. 11, 34, 46, 50, 55, 56 and 60 are reproduced by permission of the Controller, H.M. Stationery Office, and the editor of the *Journal of the Ministry of Agriculture*. The editor of *Nature* allowed the reproduction of Figs. 39, 40, 41; and the Royal Society supplied electros of Figs. 1, 3, 42, 49.

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Finally, the assistance rendered by the *Review of Applied Mycology* in enabling information to be obtained from journals, not easily procurable, must be acknowledged.

KENNETH M. SMITH.

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RECENT ADVANCES IN THE STUDY OF PLANT VIRUSES

CHAPTER I

INTRODUCTORY

Definition of the viruses and some theories as to their nature—Historical—Economic importance—Present trend of plant virus research

Definition of the Viruses and some Theories as to their Nature

It is a difficult matter to formulate any one definition which will adequately portray the very large and miscellaneous group of disease-producing agents known as viruses. There are, however, three cardinal properties which are usually considered characteristic of viruses. These are, first, their extremely small size, which is below the resolving power of the ordinary microscope, secondly, their ability to pass through the pores of a filter candle which is impermeable to visible bacteria, and, thirdly, their inability to multiply in the absence of the living cell. A definition, however, based on these characteristics is, as Dale has pointed out (8), not likely to effect a sharp or stable demarcation, and must obviously narrow its scope with the advance of technical knowledge. Indeed, improved methods of study already tend to exclude from a virus group based upon the above negative characteristics several agents which, from their general behaviour, obviously belong to the viruses. Thus the work of Elford and others with collodion membranes shows that there is no natural limit of filterability, and that viruses can be filtered or held back according to the grading of the membrane. Again, Barnard, using ultra-violet light, has photographed with his ultra-microscope certain particles, much below the range of the ordinary microscope, which are widely considered to be the actual virus entity. The third exclusive characteristic of a virus, viz., inability to multiply in the absence of living host cells, is challenged by the work of Eagles and Kordi (9), who appear to have demonstrated the cultivation in a cell-free medium of the virus of vaccinia. It is true that the work of Barnard and of Eagles and Kordi applies only to the animal viruses, but there is no reason to suppose that similar advances will not also be made in the study of the viruses affecting plants.

In place of the three negative characteristics of viruses mentioned above, *i.e.*, invisibility by ordinary microscopical methods, failure to be retained by bacteria-proof filters and inability to multiply in the absence of living cells, Rivers (29) prefers a positive characterization of the viruses, one emphasising the intimate relation that exists between them and their host cells. The positive characteristics of viruses quoted by Rivers are as follows : " The multiplication of viruses only in the presence of susceptible cells, their regeneration and production of disease in many instances in only one species of host, the marked stimulation and destruction of cells induced by their activity which on the one hand gives rise to tumours, such as Rous' sarcoma, and on the other to vesicular lesions, such as fever blisters ; the intracellular pathology frequently evinced in virus diseases by inclusion bodies, and, finally, the lasting immunity that follows the majority of virus maladies." These characteristics do not wholly apply to the world of plant viruses, although in most cases they are partially applicable. Thus, though there is a tendency among plant viruses to have a restricted host range, there are one or two, notably the viruses of aster yellows, tomato spotted wilt and tobacco ring-spot, which appear to have an almost unlimited host range. Again, although some plant viruses do produce a destruction of the cells of the host, this cannot be solely a characteristic of the virus because the same agent on another host may produce only a mottling with no cell destruction. This point is further discussed on p. 16. As regards the inclusion bodies, although these phenomena are characteristic of viruses, yet there are many plant virus diseases, probably the majority, in which inclusion bodies have never been demonstrated. Finally, although certain animal viruses do confer a lasting immunity, others leave only a transient immunity behind them, though the former are certainly in the majority. Andrewes (1) has grouped animal viruses in regard to immunity conferred as follows: those with lasting immunity are smallpox, measles, mumps, chicken-pox, herpes zoster, poliomyelitis, yellow fever, cattle plague, swine fever and dog distemper, while those with only a transient immunity are herpes simplex, common cold, foot-and-mouth disease and influenza (?). The lasting immunity conferred by virus diseases, referred to by Rivers, does not, of course, apply to plants where, with one possible exception, no immunity is acquired.

A working definition of viruses is that supplied by Gardner (14), who defines them as " agents below or on the borderline of microscopical visibility which cause disturbance of the function of living cells and are regenerated in the process."

It will not perhaps be out of place at this juncture to introduce a short discussion on the nature of the filterable viruses and indicate the general trend of scientific opinion on this matter. The reader can then decide for himself which of the current theories on the nature of viruses is best supported by the subject-matter of this book. In considering the theories of the nature of the filterable viruses, a question that arises is whether these agents are living or non-living. The line of demarcation, however, between the living and the non-living is by no means clearly drawn, and there is not in fact any single criterion of life. There are, however, certain characters, such as the power to multiply, to adapt to altered environment and to assimilate from the surrounding medium without loss of individuality which, when present together, are characteristic of living things (3). The theory of a living agent postulates the existence of an organism either of the nature of a minute bacterium or possibly belonging to a precellular form of life. The theory of a non-living agent supposes the existence of an unorganized toxin which possesses the power of coercing the infected cells to reproduce the agent of their own destruction. The following facts which have been assembled seem to favour the biological or organismal nature of the filterable viruses. The first is the faculty for indefinite reproduction in the host, to which may be added the apparent multiplication of certain plant viruses within the body of the insect vector. Some slight additional evidence is also afforded by the "incubation period," or delay in development of infective power, of the virus in some cases within the body of the insect. For this multiplication young growing cells are especially suitable and may be quite essential. The second point is the apparently successful cultivation in cell-free media of several animal viruses, *e.g.*, vaccinia, pleuropneumonia of cattle and infectious laryngo-tracheitis of chickens. The third fact is Barnard's work in photographing the apparent virus organisms of infectious ectromelia of mice, and other viruses by means of his ultra-microscope. Fourthly, a capacity for variation and adaptation is shown by viruses; thus, they can be modified by passage through different plants or animals and can be educated to withstand disinfectants. The virus of foot-and-mouth disease has been shown to occur in different strains, where infection with one strain does not produce immunity to another strain. The fifth point is susceptibility to physical and chemical agents. All viruses are destroyed at comparatively low temperatures and many are inactivated by short exposure to alcohol. Sixthly, there is the high degree of immunity which some animal viruses stimulate within the host. It should perhaps be pointed out that immunity in animal hosts induced by viruses appears to be of a slightly different nature from the immunity induced by bacteria; the former seems to be of an absolute and even lifelong nature, while the latter can usually be broken down by sufficiently large doses of the infective agent. This view, however, is contested by Gye and Ledingham (32, p. 23), who state that no dogmatic comparative statement is warranted in view of the wide variations in this respect met with both in bacterial and virus affections.

The following points seem to militate against the theory of the biological nature of viruses and to support the view that they are unorganized toxins or of an inanimate nature. Firstly, there is the extremely small size of certain viruses; that of foot-and-mouth disease is only three or four times the size of a hæmoglobin molecule. Again, from the work of Elford and Andrewes (10) the size of the smallest bacteriophage is estimated to be 8–12 $\mu\mu$, and it is difficult to conceive particles of this size as being organized. Dale (8) suggests that it is possible that some of the agents called viruses are organisms and others relatively simple pathogenic principles in solution; but to assume at this stage such a fundamental difference among members of a group having so many properties in common would be to shirk the difficulty. The next point in favour of the toxin theory is the great rapidity of multiplication of viruses within the host. This is so rapid as to suggest a transformation of matter rather than growth by division of the substance of the virus. The third point concerns the extreme longevity of certain plant viruses in dried tissues. There is some evidence which indicates that the active principle of tobacco mosaic can remain viable in leaves dried and preserved for twenty-four years, while a claim has been made that the virus of bean mosaic is capable of remaining viable in the seed for a period of thirty years. Here again no dogmatic comparative statement can be made as certain bacteria are capable of remaining viable for very long periods. Finally, the fact that certain viruses will tolerate concentrated glycerine for considerable periods does not support the view that they are closely related to ordinary bacteria.

If a virus must be placed on a higher plane than that of an unorganized toxin, then one may consider the suggestion offered by Twort (33) that possibly an ultramicroscopic virus belongs to one or more of those forms of life which in the scale of evolution are situated between the simple enzymes and the bacteria. Boycott (5) considers that it is possible to arrange a series :—

(1) The growth-promoting substances from tissues which show indirect multiplication, but make no other suggestion of life.

(2) "Lysozyme," which would pass for an enzyme except that it can multiply.

(3) The Rous agent and the bacteriophage arise repeatedly in malignant tumours and in bacteria respectively, and may be in some sense alive.

(4) The pathogenic viruses, which represent a further step towards being wholly alive.

In his recent paper on the nature of viruses Rivers (29) draws some conclusions which may appropriately be quoted here: "The confused state of our knowledge of the viruses at the present time makes it exceedingly difficult to define the nature of these active agents. The easiest way out of the dilemma, however, would be the acceptance of the presumptive evidence that viruses are minute organisms. Yet the easiest way and the one that best fits the experiences of the day may not be the right one. Furthermore, excessive scepticism and the habit of too readily accepting presumptive evidence are equally productive of sterility. Unless viruses represent a form of life unknown, proof of their living nature would not be a striking discovery. If, however, some of them are not animate, absolute proof of such a fact would be of fundamental biological importance. Therefore, care should be exercised that immoderate scepticism on the one hand, and the mental satisfaction secured by accepting presumptive evidence on the other, do not dull our efforts to obtain a better understanding of the viruses, some of which may be minute organisms while others may represent forms of life unfamiliar to us, while still others may be inanimate transmissible incitants of disease. In any event, we are face to face with the 'infinitely small in biology,' and if there be a sharp demarcation between life and death, then scientists, investigating the nature of viruses, are working near the line that separates infinitely small living organisms from inanimate active agents."

Historical

It is of interest to consider that Edward Jenner, in dealing with smallpox and vaccinia, was concerned with characteristic virus infections long before visible bacteria were associated with disease. The possibility of the existence of excessively small invisible micro-organisms was first suggested by Pasteur on his researches on the ætiology of hydrophobia. The first actual record of a virus disease is found in the work of Iwanowski (17) in 1892, who demonstrated that the sap of mosaic tobacco plants was infectious to healthy plants after passage of a bacteria-proof filter. This work passed without recognition until seven years later when it was repeated by Beijerinck, who introduced his theory of a " contagium vivum fluidum." In the meantime Loeffler and Frosch discovered more or less accidentally that foot-and-mouth disease was not due to a visible bacterium but to an " infective principle " which was small enough to pass through a porcelain filter. These discoveries gave an impetus to investigation into other diseases of unknown origin, and since that time the study of filterable viruses has assumed a great and increasing importance. At the present moment there are more than a hundred diseases considered to be due to viruses, and they include many devastating disorders affecting plants, insects, fish, birds, domestic animals and man.

Economic Importance

In endeavouring to convey to the reader some idea of the economic importance of plant virus diseases, the writer has purposely avoided quoting fictitious losses in millions of pounds sterling per annum, and has instead gathered together some figures relating to the actual reductions in yields of important crops brought about by this kind of infection.

First, as regards the potato crop, Brown and Blackman (6) have carried out field experiments on the deterioration, due to virus diseases, of Scotch potato " seed " in England. They find that an assessment of the statistical significance of the data obtained in the experiments showed that the significant percentage drop in cropping power of the locally grown seed tubers, as compared with that of Scotch tubers, varied from 7 to 20, the average being 11·8 per cent., corresponding to a reduction in yield of about 1·2 tons per acre.

Whitehead, working in Wales (35), computes the losses in yield of the potato crop due to leaf roll to be 55·8 per cent. in 1921, 45·6 per cent. in 1922 and 52·6 per cent. in 1923. The reduction in total numbers of tubers due to leaf roll was 54·4 per cent. in 1922 and 61 per cent. in 1923.

In central and western Germany a reduction of 20–30 per cent.

from virus diseases is by no means uncommon in the potato crop. The two most important diseases are mosaic and leaf roll, of which the latter is the principal cause of damage in Germany.

An investigation carried out in Canada on the effects of mild and rugose mosaic on Green Mountain, Irish Cobbler and Bliss Triumph potatoes showed that mild mosaic required four or five seasons to render an average strain unproductive, the yield rate only being reduced 10–30 per cent. during the first year and no further appreciable losses occurring during the second and third seasons. Rugose mosaic (a virus complex) is much more destructive, reducing the yield rate from 35 to 60 per cent. in the first season and rendering an average potato strain unproductive in two or three years. According to Gardner and Kendrick (15) in Indiana, the use of leaf roll seed tubers caused a reduction of 17–75 per cent. in yield compared with 51–82 per cent. where mosaic tubers were used for seed. In some experiments in New Jersey, the yields of potatoes on an acre basis were found to be as follows: healthy, 367 bushels; mild mosaic, 291; rugose mosaic, 135; spindle tuber, 143; and leaf roll, 94 bushels. Wedgworth (34) in similar experiments finds the yields to be as follows—taking an average of two plots in 1927: healthy, 89·2 bushels per acre; mild mosaic, 63·6; rugose mosaic, 27; spindle tuber, 40·4; and leaf roll, 18·7 bushels per acre. In 1928, taking an average of four plots, the yields were: healthy, 180·2 bushels per acre; mild mosaic, 131·2; rugose mosaic, 71·3; spindle tuber, 79·2; and leaf roll, 68·9 bushels per acre.

Tests carried out by McKay and Dykstra (20) in Oregon, U.S.A., showed reductions in yield amounting to as high as 73 per cent. by weight in the case of spindle tuber, 50 per cent. with crinkle mosaic and 19 per cent. with interveinal mosaic. Folsom *et al.* (13) give details of yield reductions by potato virus diseases in Maine. Spindle tuber in the Green Mountain and Irish Cobbler varieties reduced the yield rate of completely infected stocks to 20 per cent. below the yield rate of healthy stocks of the same strains. Mild mosaic in similar comparisons in Green Mountain strains reduced the yield rate to from 8 to 15 per cent. below that of healthy stocks. Rugose mosaic reduced the yield rate 50 per cent. Each 10 per cent. reduction amounted to from 30 to 40 bushels an acre (or 11 to 15 barrels). Inferior soil reduced the yield of a healthy stock below that of a completely diseased stock on good soil and increased the injurious effects of mild mosaic.

In the writer's experience of potato virus diseases in England, the aphis-transmitted virus known as Y—which produces leaf-drop streak in a number of potato varieties—and leaf roll are the most serious from the point of view of crop production.

As regards the effects of virus diseases on other crops, Faris (11) computes the reduction in the weight of Cristalina sugar cane due to the use of mosaic seed cane in Cuba as 62·9 per cent., while the losses in the successive ratoon crops average 79·5 per cent. on a five-crop period. In two series of tests on Rio Canto clay soil the average annual losses from mosaic over a five-year and a three-year period respectively were 11 and 8 tons of cane per acre.

The mosaic disease of tobacco is responsible for considerable reduction in the yield and value of that crop. These reductions have been variously assessed at 30–50 per cent. in yield and 55–60 per cent. in value, the reduction in quality being more marked than reduction in yield.

The growing of small cucumbers for pickling in the vicinity of New York has been seriously threatened by the prevalence of cucumber mosaic, while the virus disease known as " yellows " adversely affects the trade in asters. In 1924 the yield of the sugar-beet crop in the Yakima valley in the State of Washington was reduced to one-fourth of that of a normal crop by the curly-top disease of beets (Kunkel *in* Rivers (27)), and in California in 1925, 10,000 acres of sugar-beet were abandoned for the same cause. In India the virus disease of sandal known as " spike " may reduce the value of the yield by as much as 50 per cent. Berkeley (4) has compared the yield from thirty healthy with thirty mosaic raspberry bushes and finds that the former produced 13,486 berries, or 556 oz., as compared with 10,888 berries, or 398 oz., from the mosaic bushes.

A very serious virus disease of cotton known as " leaf-curl " is spreading in Southern Nigeria (Golding (16)) and in the Sudan

(Kirkpatrick (18)), and has recently been recorded by Mathur (24) as affecting garden Zinnias in North India. This virus, which is transmitted by a species of whitefly (*Aleyrodidœ*), reduces the yield in a severe attack by ½ to 1 kantar per feddan (= 312 lb. of unginned, or 99 lb. ginned cotton per acre) (see Fig. 53).

Horticulturists do not seem to realise how very thoroughly virus diseases are becoming established among ornamental plants of all kinds. In certain types of flowering plants, indeed, a state of degeneration has set in which can only be compared to the degeneration which is found in English " seed " potatoes heavily infected with virus diseases. The following examples may be cited. Daffodil and narcissus bulbs are very frequently found affected with a severe virus disease known as " stripe " in this country and as " gray disease " in America. In the west of England irises are commonly affected with mosaic, and tulips all over the country show the virus affection known as " breaking " (see Fig. 66). Freesias in the neighbourhood of Oxford and of Reading are seriously affected with a virus disease and there has recently been described (Pethybridge and Smith (26)) a mosaic disease of the cultivated geranium which appears to be very widely spread (see Fig. 54). A good example of degeneration of a flowering plant due to virus infection is found in the case of *Lilium candidum*, the stocks of which appear to be heavily infected with a mosaic virus. Ogilvie (25) has described how a virus disease of the Easter lily (*Lilium longiflorum* var. *eximium*, Baker) known as " yellow flat," or more correctly " rosette," caused a very marked decline in the export of these bulbs from Bermuda (see Fig. 65). Dahlias, *Solanum capsicastrum*, asters, lupins and double nasturtiums are all susceptible to the virus which causes " spotted wilt " of tomato.

The most serious point about the infection of flowering plants with viruses lies in the fact that many of these plants are vegetatively propagated and the virus is thus passed on from year to year until, as with potatoes, the stocks become entirely contaminated.

There can be no doubt that virus diseases of plants are of more economic importance now than they were even a decade ago.

This can hardly be due to the appearance of new viruses but rather to the increasing spread of these agents with improved methods of transport and development of communications between countries. Added to their increased dispersal is the probability of viruses becoming adapted to new host plants. In 1928 Kunkel (*in* 27) quoted five cultivated crop plants which at that time were not affected by virus diseases. These were asparagus, cabbage, flax, oats and pineapples ; now, a few years later, two of these crops are affected by viruses, *i.e.*, pineapple yellow spot (Linford (19)) and mosaic and streak of cabbage (Clayton (7)).

The last few years have seen a slow but steady realisation of the importance of intensive study of the viruses affecting plants and animals. As regards the former, in 1927 the Imperial Agricultural Conference recommended to the British Government that funds should be allocated to further investigation of this type of plant disease. In accordance with this resolution, the Ministry of Agriculture and the Empire Marketing Board have made grants of money for the founding and maintenance of research stations in different parts of the country to investigate the problem of plant virus diseases from various standpoints. Such plant virus research stations are now in existence at Cambridge, Cheshunt, Rothamsted, in Scotland and the Irish Free State. As to viruses attacking animals, including man, it is unnecessary to labour the point regarding their importance. Such devastating affections as foot-and-mouth disease, yellow fever and typhus, to quote but a few, are sufficient in themselves to show the significance of this type of infection to mankind. In America there already exist University departments of filterable viruses, and the time may not be far distant when similar departments will be created in the Universities of this country also.

Present Trend of Plant Virus Research

There is urgent need at the moment for some lead in a definite direction of inquiry in plant virus research. Not much further progress is likely to arise from continued studies of symptomatology, to which too much attention has been given in the past. Of the methods of approach to the plant virus problem, outlined in this book, there are certain lines of study which seem to the writer important. First, an intelligible and scientific means of classification and differentiation of plant viruses is much to be desired so that the confusion which exists in certain groups of viruses may be cleared up and the repeated description of existing viruses under new names may be avoided. It is probable, however, that classification must wait upon further discoveries regarding the true nature of viruses before a stable and scientific basis for differentiation can be evolved. Again, much further investigation into the relationship which exists between the virus and the insect vector is necessary, especially as regards the specificity of insects for particular viruses and the exact organ or organs in the insect's body in which the virus is located and presumably multiplies. Then, as regards studies upon viruses in suspension in plant sap, there is the method of filtration through collodion membranes as devised by Elford, which has not yet been fully tested out on plant viruses and which cannot be properly applied without much further study on the influence of various physical factors on the filtration of plant viruses, and there are other lines of study arising out of that technique, such as the concentration and purification of viruses on impermeable membranes and the possibility of using grades of membranes to separate out suspected virus complexes. The effect of enzymes upon virus suspensions has hardly been investigated at all, and studies on these lines may also reveal the existence of virus complexes hitherto unsuspected. Attempts to cultivate the plant viruses in cell-free media should be continued, more especially in view of apparent successes obtained in this direction with certain animal viruses, and it is to be hoped that some attempts to photograph the various plant viruses by ultra-violet light may be made.

Investigations into methods of control of virus diseases affecting economic crops should be largely directed towards the production of varieties resistant or immune to the virus concerned and some progress in the production of virus-resistant crops, notably sugar cane, appears to have been made already. The problem of virus carriers is one of great scientific and economic importance, and practically nothing is known of the reasons underlying this phenomenon, nor the reactions of the carrier plant under controlled environmental conditions.

As regards entomological investigations in the field, ecological studies on the insect vectors are needed, particularly as regards the factors influencing the movements and migrations of such insects.

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CHAPTER II

SYMPTOMATOLOGY

Appearance of virus-affected plants—Pathological histology—Effect of environment upon the symptom picture.

The symptomatology of virus-affected plants is here discussed under three heads. First, the symptom expression or " clinical picture " exhibited by the plant as a whole is dealt with as the " appearance of the affected plant," secondly, the microscopical changes taking place within the tissues are described under the heading of pathological histology and, thirdly, the effect of the environment upon the symptom picture is discussed.

Appearance of Virus-affected Plants

Although the symptoms induced in plants by viruses are very varied in character, yet there is an indefinable similarity which links them together, and this is especially true in the " mosaic " group. This general similarity is no doubt partly due to the systemic invasion of the host by the virus, which induces evenly distributed, rather than localized, symptoms.

For convenience of description the symptom pictures of virus-affected plants may be broadly classified into groups. First, there are those diseases in which the chief symptom is a chlorosis or mottling of the leaves ; these are known as mosaic diseases and may or may not be accompanied by leaf distortion. Secondly, there is a type of necrotic disease in which a destructive element predominates and where necrotic spots, streaks or stripes may occur on the leaves and stems of the affected plants. Thirdly, there is a large and miscellaneous group of virus diseases where the chief symptom exhibited consists of a distortion or growth stimulation of the whole or part of the affected plant. In this group may be placed such diseases as " alloiophylly " of anemone, cranberry "false-blossom," "spike" of sandal, "giant-hill" and leaf roll of potatoes, the "Fiji disease" of sugar cane and many others.

In discussing the symptoms of virus diseases in animals, Rivers (43) suggests that there exist two main types of disease, in which two forces seem to operate, the one stimulating the cell, the other destroying it. Consequently the symptom picture produced by an animal virus disease is more or less dependent upon which of these two forces predominates. Rivers quotes, among others, chicken-pox, foot-and-mouth disease and bacteriophage as examples in which the destructive agencies predominate, and Rous sarcoma and warts as examples in which stimulating forces are dominant. It may perhaps be permissible to apply this concept to the virus diseases of plants where these two forces also appear to operate to a certain extent, the destructive agent being the more important. In certain cases, however, the same virus may be capable of producing both effects according to the species of host plant, *i.e.*, a destructive necrosis on one plant and a proliferation on another. This fact is illustrated by a virus of tobacco mosaic which produces a systemic necrosis in *Nicotiana rustica* and a proliferation in the leaves of *N. tomentosa* (22) and *N. paniculata* L. (24). Nevertheless, as above mentioned, there do occur viruses where the main force is destructive, producing a severe necrosis of stem or leaves ; many of the mosaic viruses are included in this group. This destructive element, however, may only develop when the virus affects a specific host.

The stimulating force may be said to occur in those diseases which produce galls, excrescences on the veins, overgrowth, elongation of tubers, abnormally large plants, etc.

The existence in the mosaic viruses of a third force, which is inhibitory rather than destructive or stimulatory, must be considered. Thus in plants of *Solanum nodiflorum* and *Nicotiana tabacum*, affected with the virus of aucuba mosaic of tomato, certain of the leaf tissues are devoid of plastids and the cells may be undifferentiated. The absence of chlorophyll is brought about by the inhibition by the virus of the development of the plastid primordia. If plastid development is not prevented in a very early stage, perfectly normal plastids are formed. It is thought, therefore, that the virus inhibits production of, rather than destroys, the chlorophyll (48).

Owing to the facts that plant viruses have not yet been cultivated in cell-free media and are not amenable to the methods of differentiation applicable to bacteria, the symptom expressions of plant virus diseases have been their chief means of differentiation, and have thus received an amount of attention somewhat disproportionate to their importance. Nevertheless, unreliable and misleading as they are, symptoms cannot be dispensed with as an aid to diagnosis and must continue to be an important factor in plant virus research. For this reason, therefore, it is proposed

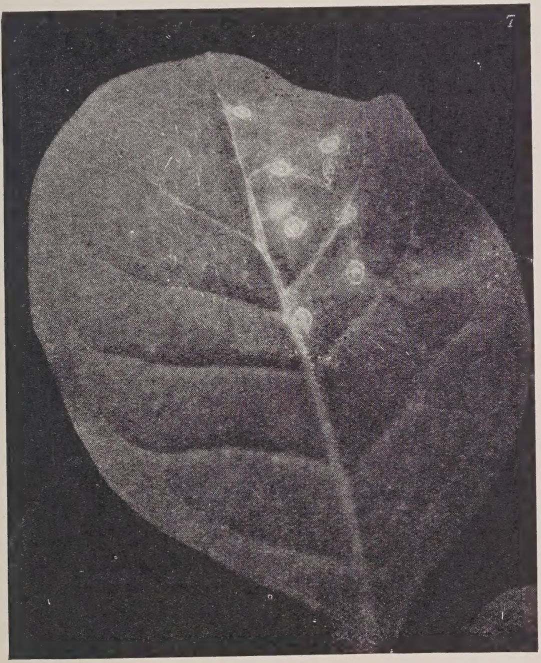

FIG. 1.—Primary symptoms of the potato virus X cn the tobacco plant, var. White Burley. Note the rings forming on the inoculated leaf.

to deal in some detail with the more common types of symptoms exhibited by plant virus diseases.

In many cases, particularly as regards mosaic viruses, there occur two phases in the development of the disease. These phases are designated the *Primary* and *Secondary* symptoms; the primary symptoms develop before the virus is generally distributed throughout the plant, while the secondary symptoms are the result of systemic invasion. Thus the primary symptoms frequently take the form of local lesions, spots or rings upon the inoculated leaf (see Figs. 1, 2) according to the virus, and these are followed shortly after by the development of the general symptom picture. For example, in the case of the potato virus known as Y (53) the primary symptoms induced by this virus on tobacco and certain other plants consist of an intensification or clearing of the veins of the younger leaves (see Fig. 3). This symptom rapidly disappears and is followed by the expression

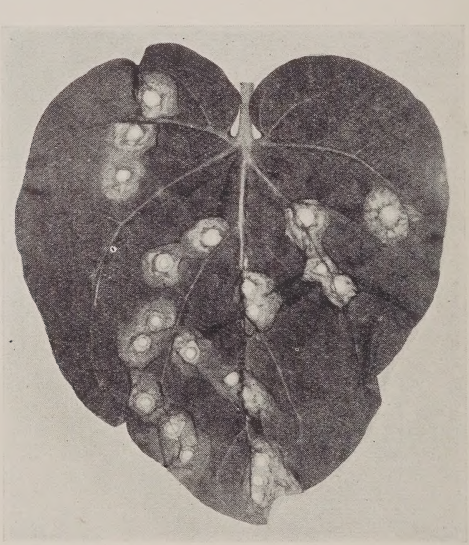

FIG. 2.—Primary lesions of the virus of tomato spotted wilt on *Nicotiana glutinosa* nine days after inoculation. (After Samuel and Bald.)

peculiar to systemic invasion by this particular virus. It may be stated here that this preliminary " clearing of the veins " is a characteristic primary symptom of several plant virus diseases, and it occurs, in addition to the case of the Y virus, here mentioned, in aster yellows, curly-top of sugar beet and in many of the tobacco mosaic diseases. The primary symptoms of leaf roll on potato take the form of a rolling of the younger leaves only ; this is followed by a rolling of the lower leaves as the virus becomes distributed. It should be noticed that the same virus does not always produce the same primary and secondary symptoms on different plant species.

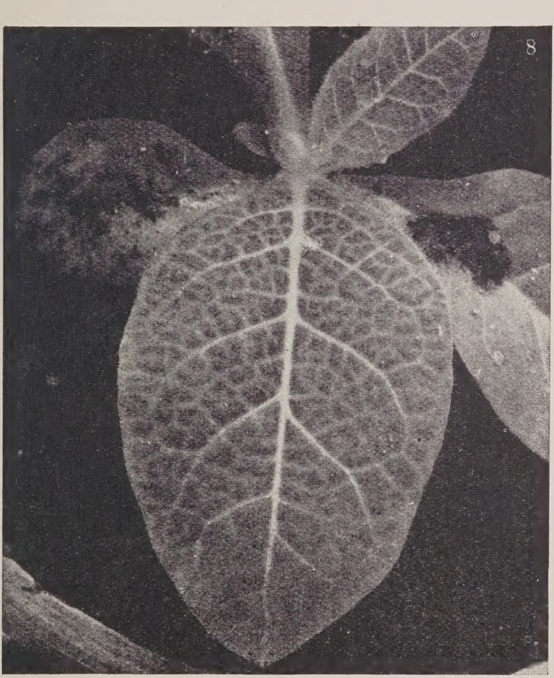

FIG. 3.—Primary symptoms of the potato virus Y on the tobacco plant, var. White Burley. Note the " clearing of the veins " of the younger leaves.

As regards the rather miscellaneous group of mosaic viruses, it is noteworthy that the type of chlorosis differs considerably, not only among the various mosaic viruses, but in the same virus on different plant hosts. The mildest type of mottling occurs among some of the mosaic viruses affecting the Solanaceæ, particularly potato and tomato. Here the symptoms take the form of a mottling of differing shades of green, which may be so faint as to be almost indistinguishable. It does not follow, however, that the virus itself is innocuous to all hosts because of the mildness of its symptoms on certain plants. This is well illustrated by "mild" mosaic of the tomato, which produces, as

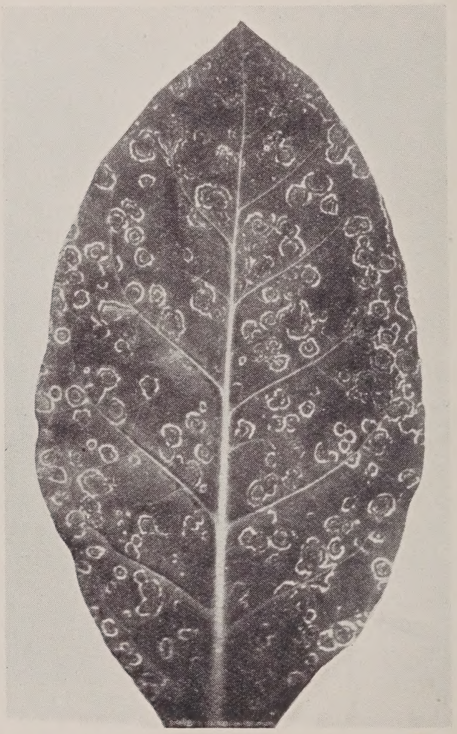

Fig. 4.—Potato virus X on tobacco, var. White Burley: an example of a "ringspot" virus, in which the chief symptoms take the form of numerous rings.

its name implies, a faint mottling only on that host. Transfer, however, of this virus to the tobacco plant induces in that host a fierce and often lethal necrosis of the stem and petioles (54). Another case in point is the potato mosaic virus, known as X (53); the commonest symptom expression of this virus on the potato is a faint mosaic mottling, but on tobacco it usually appears in a very characteristic and well-marked form of concentric necrotic rings (see Fig. 4). This development of numerous concentric rings with a central spot, or Liesegang phenomenon, as it is called (18), is shown by several mosaic viruses which, when in this form, are known as " ringspot " viruses. The rings are usually necrotic, but may be chlorotic; they occur on the leaves, and, less commonly, on flowers and stem (see Figs. 4, 5).

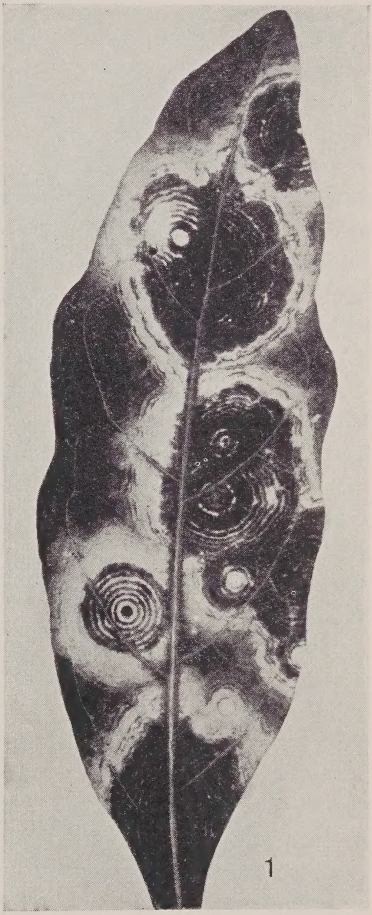

FIG. 5.—The virus of tomato spotted wilt on *Solanum capsicastrum*, an example of a " ringspot " virus. Note the large numbers of concentric rings with a central spot.

A more pronounced form of chlorosis is exhibited by the tobacco mosaic viruses. On tobacco leaves the symptom expression consists of unusually bright patches of green, yellow or sometimes white, according to the virus (see Fig. 6); such diseases are known as " yellow tobacco mosaic " and "tobacco calico." These viruses also have an alternative expression in the form of a severe necrosis on tobacco and other plants.

There are other mosaic diseases which express themselves in various spottings, specks and streaks upon the leaves and sometimes on the fruit and flowers. A good example of symptoms induced upon the flower by a mosaic virus is given by the disease of tulips known as "breaking" (see p. 393). In this disease the flowers become variegated to bi-colours, the original self-colour or a darker shade of the same being restricted to splashes or lines somewhat irregularly distributed on a white or yellow ground as the case may be (8). Many of the attractive variegated tulips now to be seen are merely mosaic-diseased specimens of a uniform self-coloured variety. Thus the valued Rembrandt variety consists of diseased specimens of the variety Princess Elizabeth. In America the claim has been made that it is possible to produce "new" varieties at will by the simple process of inoculating the plants with one of these "breaking" viruses.

Other examples of flower symptoms may be found in the gladiolus and on species of *Nicotiana* affected with mosaic and occasionally on zinnias affected with the virus of tomato spotted wilt.

The bright variegations exhibited by the leaves of *Abutilon striatum* Thomsoni was shown by Baur (2) to be due to virus infection, or, as he named it, "chlorosis infectiosa," transmissible only by grafting. This infectious chlorosis behaves similarly to many other mosaic diseases in that it is capable of producing a destructive necrotic effect when transmitted to another plant species, in this case *Abutilon indicum*.

There are thus three possible forms of symptom expression in mosaic diseases : firstly mottling, stripes or streaks of light, dark green, yellow or almost white upon a background of darker green ; with these symptoms are often associated in varying degrees curling, crinkling, blistering or other malformation of the affected leaves. Secondly, there is the development on the leaves of numerous single circles or concentric rings, usually with a central spot ; and, thirdly, there is the necrotic destruction of the tissues in leaves and stem. In certain cases, as exemplified by the virus causing spotted wilt in tomatoes, a single virus is capable of inducing all three types of symptoms according to the species of plant host, the duration of the disease and the age of the plant affected. The expression of symptoms in mosaic viruses is thus correlated to a high degree with the species of plant host, and this is especially true of potato mosaic viruses and their expression in different potato varieties.

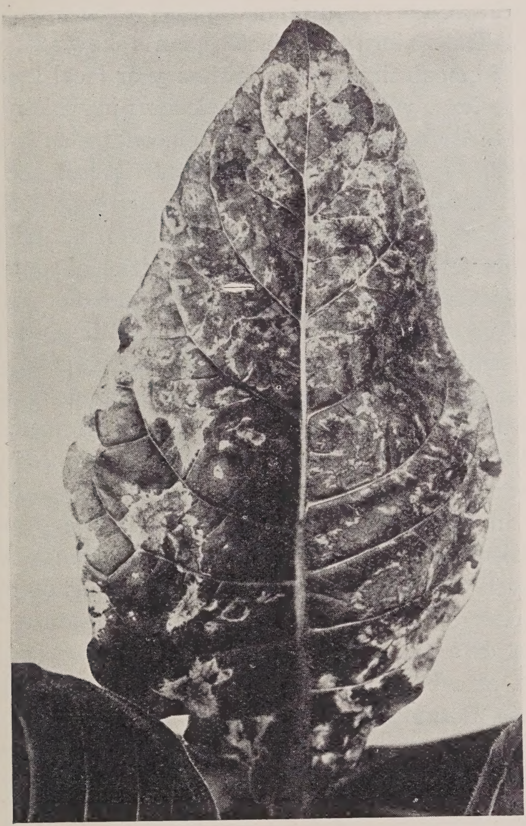

FIG. 6.—Tobacco mosaic. This is a good example of mosaic mottling.

The very wide range of symptoms exhibited by one virus is well illustrated by some recent work by Holmes (22), who has studied the effects of " ordinary " field tobacco mosaic upon a wide range of hosts. The following is a brief description of the primary and secondary symptoms of this virus upon a range of plant hosts. The majority of infected plants show some symptoms at the site of inoculation. These primary or local lesions consist of necrotic lesions, yellowish lesions, lesions in which the chlorophyll is retained abnormally long under conditions favourable to the loss of this pigment from uninfected tissues, and lesions in which the chlorophyll is retained abnormally long during storage in darkness. All these lesions are similar in form, and apparently represent interference with normal function in spots in which virus is increasing. Abscission of an inoculated leaf also occurs as a primary symptom of infection. In addition to the above-mentioned primary symptoms most hosts of this virus show secondary or systemic symptoms. The commonest of these are mottling, leaf distortion, stunting of plants, necrotic secondary lesions, scattered yellowish spots and lesions invisible in the living plant but demonstrable by staining with iodine after holding the plants in darkness. Less common systemic symptoms are abnormal tissue outgrowths, defoliation, loss of flowers and fruit, prolonged yellowing, bending of stems, spotting of flowers and failure to remove chlorophyll from secondary lesions.

The virus of a tobacco mosaic, like that of tobacco leaf curl (see p. 372), exercises a stimulatory action on growth in certain plant species, notably *Nicotiana tomentosa*, Ruiz. and Pav., and *N. paniculata* L., and produces leaf-like outgrowths (enations) on the undersides of the leaves. These outgrowths develop only on leaves produced after systemic infection with the virus. It appears that these leaf enations are produced only when the virus is present in leaves at the time of their early development (24).

There is another symptom picture which, although of the mosaic type, is sometimes put in a separate category and designated " yellows." Here there is no mottling but a general chlorosis or yellowing of the whole leaf. Examples of this kind of virus disease are aster- and peach-yellows.

Several of the mosaic viruses may give rise to symptoms on the stem. Thus the virus of tomato spotted wilt frequently causes a severe necrosis in the stems of tobacco plants and occasionally produces similarly two or more concentric rings. The virus of mild tomato mosaic causes a severe necrosis of the stems of tobacco plants and tomato stripe gives rise to stem lesions on that plant from which the popular name of the disease is derived.

Turning now to a consideration of the other group of diseases, it will be found that chlorosis and necrosis have given place to deformity and abnormal growth of the whole or part of the affected plant. Stunting and rosetting are common deformities and are found in such diseases as bunchy-top of bananas, rosette disease of ground-nuts, stunt of rice, curly-top of sugar beet and others which, like the foregoing examples, take their names from the most pronounced symptom of the disease. In the rosette diseases the internodes of the stem are shortened, the leaves being thus brought close together and the whole plant stunted and dwarfed.

As mentioned in a previous paragraph, there is a group of plant viruses in which the predominant factor is stimulatory of the cell rather than destructive, and a few examples of this abnormal growth are given here. In the Fiji disease of sugar cane, elongated galls are produced on the affected stems and leaves, and galls are also produced in a virus disease of anemones known as " alloiophylly." Another type of overgrowth is exhibited by the " spindle-tuber " disease of potatoes in which the tuber is longer than normal and somewhat spindle-shaped. Verplancke (58), by measuring the length-to-breadth ratios of the cells in healthy and spindled tubers respectively, considers that he has proved this elongation to be a specific result of the action of the virus. The so-called Giant Hill disease of potato plants in America, possibly synonymous with the European " bolter," appears to be another type of overgrowth induced by stimulatory virus infection.

It will be seen from the foregoing examples that external symptoms of plant virus diseases occur mainly on the leaves—less frequently on stem and flowers. In certain cases the fruits of affected plants may also develop symptoms—for example, the virus of cucumber mosaic produces mottling and excrescences on the fruit, while the fruits of tomato plants affected with spotted wilt frequently exhibit numerous concentric rings. The virus of tomato stripe also causes the development of " greasy " lesions on the green fruits (see Fig. 60). Apart from the visible symptoms, the fruits may be adversely affected by the virus in that they are tasteless and poor in quality, as in peach-yellows for example.

With one or two exceptions the roots of plants appear to be but little affected by virus diseases ; in spike disease of sandal, however, the finer roots die prematurely, and similarly the root systems of sugar beets infected with curly-top are dwarfed and abnormal. In the " phony " disease of peach the virus appears to be located only in the roots.

Pathological Histology

Under this heading will be described the chief microscopical changes which have been observed to be characteristic of virus-affected plants.

Microscopical examination of such diseased plants reveals two main kinds of pathological conditions. There are, first, the more obvious necroses and tissue alterations wrought by the virus and, secondly, certain intracellular inclusions, among which are those usually known as X-bodies. The last named will be dealt with first. Considerable controversy has arisen over the nature of these cell inclusions, and much investigation has been carried out upon them in potatoes and other plants. X-bodies appear to be peculiar to virus diseases—they are found in both living and fixed material and occur in animals as well as plants. It is proposed, then, first to give a short description of these inclusions and the particular viruses with which they are known to be associated, and then to discuss their aetiological significance and their possible relationship with the cell inclusions associated with animal viruses.

A typical X-body is a mass of material which is not unlike protoplasm in appearance, granular or very finely reticulate in structure, and usually staining more or less deeply with aniline dyes (see Figs. 7, 8). It is well defined and sometimes appears to have a definite membrane or bounding wall. In shape it is rounded, often roughly spherical, but frequently elongated, and the larger forms may be constricted or bent or of almost any outline, while still retaining the rounded contours wherever the body has any considerable mass. Typically the body is vacuolate, with sometimes only one large vacuole, sometimes as many as ten or more small vacuoles, of which all may be scattered through its substance or some may lie at the periphery; the margins of the vacuoles often stain more deeply than the rest of the mass. Inside each vacuole may sometimes be seen a small particle with radiating threads; and in the substance of the body is regularly, though not invariably, a varying number of small deeply staining

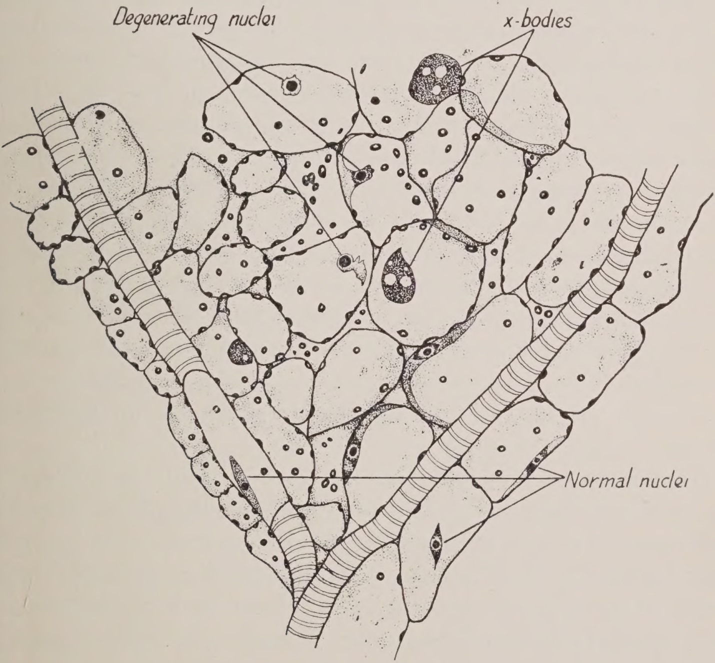

FIG. 7.—X-bodies in mosaic (= virus X) potato leaf. Section, 8 μ thick, cut in the plane of the leaf. (Flemming's triple stain.)

rounded or angular particles. The whole may have a size which varies in different cases from about 3–4 μ up to 20–25 μ in diameter, and in the elongated forms sizes of 35 μ or upwards in the longer axis are recorded. There may be only one in a cell, and this is perhaps the most usual number, but on the other hand there may be as many as ten and eleven bodies, and these possibly of different sizes, inside a single cell. They tend to be associated in position with the nucleus, lying in close contact with it, sometimes enveloping it : but they are never incorporated with it, and, when carried in the protoplasmic streaming, may be seen to impinge on it, sometimes denting it temporarily and sometimes being indented by it (Henderson Smith (19)). Only one case of an intranuclear body in plant cells has been recorded, and this has been described by Goldstein in mosaic of dahlia (16). It has been demonstrated by chemical tests that the bodies in *Solanum nodiflorum* affected with tomato aucuba mosaic are of a proteid nature and give negative fat reactions (20). Mitochondria occur in the X-bodies and several workers have observed particles, elongated or rod-shaped, within the vacuoles, but there are no structures within the X-body which could be interpreted as nuclei.

Iwanowski in 1903 (23) was the first to make a histological study of a virus-affected plant, and he described plastic amœboid bodies in the cells of mosaic-affected tobacco leaves. Seven years later Lyon (32) described small spherical and irregularly shaped bodies in the gall cells of sugar cane affected with Fiji disease. In 1922 Kunkel (28) reported similar X-bodies as being associated with the mosaic disease of *Hippeastrum equestre* Herb. In 1924 the present writer described and figured these characteristic cell inclusions in the chlorotic areas of leaves from mosaic potato plants and put forward the view that they were degeneration products of the cell induced by the virus. Since then a great deal of work has been carried out upon the intracellular inclusions of Solanaceous plants infected with virus diseases, and this work is shortly reviewed herewith.

In 1927 Hoggan (21) published an account of her histological studies upon the virus diseases of Solanaceous plants. She examined microscopically the effects of a number of viruses upon various Solanaceous hosts, including the potato. Hoggan found that typical X-bodies were a constant feature of tobacco mosaic upon a number of hosts which develop definite symptoms of mottling or chlorosis. Similar inclusions are associated with two other virus diseases of Solanaceous plants, *i.e.*, yellow tobacco mosaic and medium tobacco mosaic ; these two viruses may be closely related to the virus of " ordinary " tobacco mosaic. Hoggan also found that eight other virus diseases produced no cell inclusions in any of the Solanaceous hosts examined ; these are cucumber mosaic, speckled tobacco mosaic, mild tobacco mosaic, spot necrosis (since shown to be a virus mixture), ring-spot (cf. Woods below), bleaching mosaic, tomato stem necrosis and petunia mosaic. In addition, inclusions were not observed in potato leaf roll, streak and potato " calico." In 1930 Henderson Smith (20) described the cell inclusions found in *Solanum nodiflorum* affected with the yellow or aucuba mosaic of tomato. He found that *S. nodiflorum* was an unusually suitable host for the study of particular cell inclusions owing to the fact that the X-bodies were exceptionally conspicuous in the cells of the leaf hairs and ordinarily occurred unaccompanied by other abnormal cell inclusions, such as striate material or crystals, which, in tobacco for example, tend to complicate the picture. Finally, Clinch in 1931 published an account of her histological studies upon potato plants affected with certain virus diseases (12). She found the typical X-bodies in the chlorotic areas of the leaves in association with simple mosaic, crinkle (a composite disease) and streak ; they were not found in aucuba mosaic or leaf-roll plants. Clinch found the X-bodies to be most abundant in interveinal mosaic and crinkle plants suffering from the prolonged effects of the disease. They were inconspicuous in young diseased plants. The conclusions arrived at by Clinch agree fairly closely with those of other workers in the same field.

The following is a list of plants, other than those discussed above, affected with mosaic diseases in which the X-bodies have been observed : petunia, henbane, pepper, raspberry, pea, bean, clover (Dickson (13)), pecan (Rand (41)), sugar cane (Cook (9),

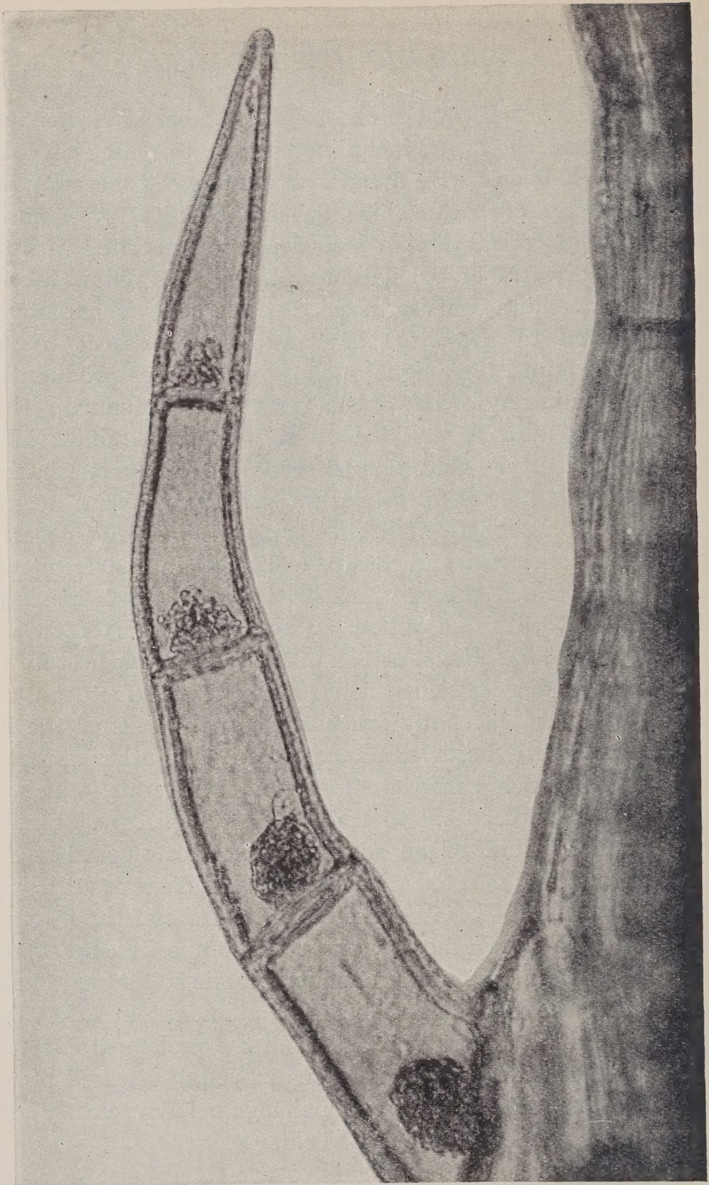

FIG. 8.—*Solanum nodiflorum* infected with "aucuba" mosaic of tomato: hair showing X-bodies. Carnoy fixation, unstained, × 430. Note the granular appearance; and in the two terminal cells the projecting particles. In the third cell the nucleus is visible at the upper edge of the body. (After Henderson Smith.)

Matz (34)), *Datura stramonium*, pokeweed, and Aquilegia (51), Chinese cabbage (29), dahlias (16), tobacco ringspot (Woods (60)).

It will appear from the foregoing account as if these cell inclusions are chiefly associated with mosaic viruses ; while this is partially true, they have also been described in connection with other types of viruses. Thus they are found associated with the peculiar " alloiophylly " of anemones (26) ; in spike of sandal (36), in rosette of wheat (33), and in the Fiji disease of sugar cane (32). They are not associated with the potato virus Y or with potato leaf roll.

Ætiological Significance of X-bodies. Although the majority of workers are agreed that X-bodies in plants are protoplasmic in nature, there has been until comparatively recently a considerable divergence of opinion as to their exact significance. Certain observers, including Sorokin (50), Kunkel (27), McKinney, Eckerson and Webb (33), Lyon (32), Goldstein (16) and Likhité (31), have inclined to the opinion that these vacuolate inclusions were either the ætiological agent or intimately connected with it. On the other hand the following workers consider that these bodies are more probably a product of the host cell cytoplasm, due to the action of the virus, though the possibility of the causative agent being contained in the X-body is recognized : Iwanowski (23), K. M. Smith (52), F. F. Smith (51), Plakidas (38), Hoggan (21), Henderson Smith (19), Sheffield (47) and Clinch (12).

Clinch has shown that there are numbers of fat droplets adhering to or embedded in the cytoplasmic mass of the X-body. These fat globules appear to arise from the breakdown of the lipoid-protein complex of the cytoplasm as a result of the pathological condition of the cell. Even in the early stages there appears to be an abnormal amount of fat in the cytoplasm of the diseased cell.

The facts which seem to lend some colour to the theory that the vacuolate inclusions are the actual ætiological agent, or at least of a living and independent nature, have been well summarized by Henderson Smith (19) and are as follows :—

(1) The general amœba-like appearance, with which may be included the pseudopodium-like projections which suggest amœboid movement.

(2) The appearances suggesting fission and the occurrences of several bodies in the same cell which might be due to division.

(3) The increase in size.

(4) Their protein nature and the presence of mitochondria.

Recent work, however, has tended to confirm the view, already most generally held, that the X-bodies in plants are to be interpreted as a reaction of the infected cell to the presence of the virus. Henderson Smith and Sheffield (20, 47) have observed the gradual building up of the X-body in living hair cells of *Solanum nodiflorum* infected with the virus of tomato aucuba mosaic. Sheffield noticed that in the early stages of infection there appeared in the streaming cytoplasm of the hair cells small particles which were carried along in the stream and tended to aggregate together to form larger masses. It has thus been found possible to follow in the individual living cell the formation of the X-body from its early beginnings to its completion. The building up of the X-body in the cell of a virus-affected plant appears to take place in the following manner. Very small particles appear in the cytoplasmic strands and are carried round the cell in the stream. These particles increase in size and tend to be arrested for a time at the anastomoses of the strands before they move on once more. With still further increase in size these halts become longer, and one or more particles may join the stationary one and sometimes fuse with it, forming a larger mass recognizable as an X-body. These masses thus formed may remain permanently in union or separate once more into their constituent parts, thus giving rise to what appears to be a process of fission. It may be suggested that this mode of formation accounts for many of the appearances which have been considered to support the belief that X-bodies are living entities, such as, for example, the pseudopodia, the fission figures and the occurrence of several bodies in one cell which has been attributed to division of a single parent body.

The small particles are considered to be foci where the protoplasm has condensed or solidified and are of no fixed shape. There is no sign of autonomous movement and nothing to suggest that either they or the completed X-body is living, in the sense of being an independent organism or parasite. This view is further supported by the fact that the X-bodies tend to crystallize out, especially in older leaves, and the crystals, thus formed, appear to have all the characters of protein crystals.

The position is summed up by Henderson Smith as follows : if this mode of formation is confirmed by other workers there would seem little reason to believe that the X-bodies are themselves organisms or stages in the life-history of the virus parasite. They should rather be looked on as a reaction of the cell cytoplasm, and it may be that each of the tiny particles observed in the early stages is evidence of a localized reaction to an ultra-microscopic particulate virus embedded in its centre.

Some further evidence on the inanimate nature of these X-bodies is afforded by a recent statement in *Nature* (Sheffield (49)), which describes for the first time the artificial production of X-bodies in plants in the absence of a virus. With molybdenum, given in the form of molybdic acid, or its ammonium or sodium salts, it was possible to induce, in the cells of *Solanum nodiflorum*, processes analogous to all stages of an attack by the virus of tomato aucuba mosaic. After treatment with molybdenum a single large mass or X-body was gradually built up by aggregations and successive fusions. The body, as finally formed, was rougher in outline and slightly more granular than those produced by infection with the virus of aucuba mosaic, to which it appeared to be essentially analogous.

It will be instructive briefly to compare the X-bodies of virus-affected plants with the apparently similar intracellular inclusions associated with many animal viruses and to endeavour to draw analogies between them. Intracellular or cytoplasmic inclusions have been observed in the following diseases among others : the Negri bodies of rabies, the Guarnieri or Paschen bodies of vaccinia, the Bollinger bodies of fowl-pox and the Borrel bodies of sheep-pox. Similar inclusions have been observed in psittacosis and infectious ectromelia of mice. As is—or perhaps was—the case of the X-bodies associated with plant viruses, there seems to be a good deal of uncertainty as to the exact nature of the cytoplasmic inclusions found in the animal virus diseases, but a strong current of opinion is setting in which favours the view that the latter may be or may contain the actual ætiological agents of the diseases in certain cases.

It must be admitted that such cytoplasmic inclusions as the Bollinger bodies or those described by Ledingham (30) and Barnard (1) from infectious ectromelia of mice do not appear at first sight to be comparable with the X-bodies of plant viruses. Ledingham describes the Bollinger bodies of fowl-pox as agglomerations of elementary bodies (0·25 μ in diameter) enclosed in a lipoprotein matrix. It has been shown that a single Bollinger body, when washed with saline and inoculated into the skin of a hen, has produced a typical fowl-pox lesion containing the characteristic bodies. Woodruff and Goodpasture (59) conclude from this that these inclusion bodies are true virus bodies, *i.e.*, minute colonies of the ætiological agent of the disease. There appear to be similar elementary bodies comprising the cytoplasmic inclusions of infectious ectromelia of mice, and Barnard describes these as follows: the virus produces typical inclusion bodies in the cytoplasm of epithelial cells which, when fully developed, are oval bodies about 12–13 μ in length and 6–7 μ broad. Barnard considers that there is no evidence of a limiting membrane of any kind. The natural bodies as seen in fresh sections appear to behave like colonies of organisms, growing rapidly for a short time, until a certain maximum dimension is reached. At the earliest stage in its formation at which an inclusion body has been recognized as such, it is spherical in shape. As it enlarges and approaches maturity it becomes somewhat elongated. In such a body photographed under these conditions it is easy to see that the substance contains refractile granules of uniform size in large numbers, but not so thickly set as to prevent their being resolved by the microscope. These minute particles, identical in size and appearance, have been found closely packed in the homogeneous matrix of the characteristic inclusion body and free in the infective extracts from organs in which inclusion bodies are not present. Barnard considers that this uniformity of arrangement strongly suggests that which occurs in a colony of bacteria.

Some recent work on the virus of psittacosis throws further light on the nature of the intracellular inclusions associated with animal virus diseases. Bedson and Bland (4) have observed what they consider to be a developmental cycle of the psittacosis virus. Mice were employed in the work and, at intervals after inoculation, these animals were killed and a study of the virus made. It was found that there were stages in an apparent developmental cycle, and these stages were linked not only in morphological sequence, but also in time, and the different forms could be obtained more or less at will, simply by killing the mouse at the requisite moment. The stages in this developmental cycle consisted of the following forms: (1) The elementary bodies, which appeared to be the last stage; (2) amœboid forms, which seem to coalesce, forming large plaques; (3) and later, smaller spherical bodies packed with oval segments. Two possible explanations of this cycle of changes are offered. One is that the elementary bodies, having reached a suitable environment in the host's tissues, proceed to multiply and to give rise to the large forms. These in turn divide, and the virus becomes progressively smaller until particles the size of the elementary bodies are again reached. The large plaque forms could only be explained on this theory by supposing that they were virus colonies surrounded by a sheath of homogeneous material. This would then be similar to the inclusion body of infectious ectromelia of mice, but would differ from it in being basophile instead of acidophile and in the fact that the virus particles were invisible because of the enclosing sheath. The alternative explanation, preferred by Bedson and Bland, is as follows: the elementary bodies, having gained susceptible cells, develop into amœboid forms which coalesce to form a plasmodium. This plasmodium then divides into a number of portions of equal size which might be termed the morula stage; these oval segments then divide and subdivide, producing once more the elementary bodies. Such a cycle, elementary body→amœboid forms running together to form a plasmodium→morula→division and subdivision of elements of morula→elementary body, would imply that the virus of psittacosis is not of the nature of a bacterium, but possesses affinities with the mycetozoa or myxomycetes, or possibly with the microsporidia.

Taniguchi *et al.* in Japan (55) describe an apparent virus organism which they consider to be the causal agent of smallpox. It is a short coccoid body measuring 0·3 μ in diameter. Some are in diplococcoid form or in chains composed of three or four bodies. There is sometimes a halo round the " organism " ; motility was not observed. In general they are nearly globular ; some are pointed at one end and some have flat surfaces, while others show an irregularly shaped and lightly staining periphery with a deeply staining centre. The same workers (56) have described a similar " organism " as the ætiological agent of chicken pox. The bodies are of isolated coccoid form, nearly the same as in smallpox but arranged like diplococci.

While it is true that the plant virus X-bodies do contain a certain number of particles, they are not comparable to the large numbers of equal-sized particles observed in the animal inclusion bodies, and it seems questionable whether the two kinds of cytoplasmic inclusions are really analogous.

As regards the Guarnieri bodies, Cowdry (*in* 43) states that these are dissolved by 1·5 per cent. acetic acid, which is not the case with X-bodies associated with potato viruses (Clinch (12)). Further, the vaccinia bodies are soluble in dilute acid, thus indicating an albuminous nature, and do not give a positive Millon's reaction, while the X-bodies from virus-affected *Solanum nodiflorum* give all the usual protein reactions—Millon, biuret, etc. (20).

According to Cowdry (*in* 43) the mitochondria are rarely directly involved in the differentiation of animal virus inclusions in the sense of actual transformation. In most cases it is the ground substance that is concerned. Some of the inclusions hug or indent the nucleus, others do not ; this phenomenon also occurs in the case of plant X-bodies. Evidently the ground substance of the cell is generally equipotential, or nearly so, in its capacity to elaborate the inclusion.

Using Borrel's coloration technique (*Bull. de Dermatol.*, 9, 1930), Dufrenoy states that he has detected, in the epidermal cells of mosaic tobacco plants, bodies analogous to those found by Borrel in the tissues of animals suffering from virus diseases.

The cytoplasm is stated to show pink or red spots occupied by numerous minute, sharply defined, homogeneous granules which were not found in normal green cells (14).

No discussion is given here of the intranuclear inclusions common in animal virus diseases because there appear to be no comparable bodies found in plant virus diseases, except a single instance described by Goldstein (16).

The present attitude then towards the X-bodies of plants may perhaps be summed up in the hypothesis that these intracellular inclusions are formed as a reaction to stimulation by the virus, which, however, as suggested by Henderson Smith, may be located within the smaller particles observed in the early stages of the formation of the larger inclusion body. On this assumption the intracellular inclusions of plant virus diseases are not analogous with the cell inclusions of ectromelia and other animal viruses if the hypothesis is accepted that the elementary bodies contained within the larger body are the actual causative agents.

Before dealing with further histopathological changes which are associated with plant virus diseases, mention must be made of another cell inclusion which appears to be distinct from the X-bodies above discussed. This type of inclusion is known as "striate material," and has been described in association with potato and tobacco mosaic, among others, by Hoggan (21) and Rawlins and Johnson (42). This "striate material," though abnormal in shape, appears to be similar in structure to what occurs in normal cells. The striate material is usually found in contact with the nucleus, and there may be some relation between the two. Owing to its amorphous nature it is likely that the striate material is a product of the diseased cell. Iwanowski (23) and Goldstein (16) consider that it arises from the action of acids in the fixing fluids on certain waxy-crystalline plates occurring in the living diseased cells. The striate material, however, is easily visible in masses in the living cell and the striation is accentuated by the fixation.

Turning now to the examination of other histopathological changes produced in plant tissues by viruses, it will be convenient to consider first the modifications underlying the mottling effects in mosaic-infected leaves. Clinch (12) finds that the extent of these modifications is proportional to the disease symptoms and draws attention to the disagreement that exists among workers as to the nature and extent of the modifications which underlie the mottle effects in mosaic-infected leaves. Various workers have pointed out the lack of proper differentiation and development of the cells in the chlorotic areas of mosaic-infected leaves and have referred to the smaller size and paler colour of the plastids in the chlorotic areas. Cook (10) has studied the effect of mosaic on the cell structure and chloroplasts in a number of different plants, among which are included tobacco, tomato, sugar cane, cowpea and pepper. He considers that the the effect on the cells of the mosaic viruses in these and other hosts is purely inhibitory and prevents the formation of the chloroplasts. Cook states that it was possible to distinguish at an early stage, in some hosts, those areas which were to become chlorotic from the future green ones. In the former the cells were undifferentiated and the chloroplasts few and small, while in the latter the palisade was slightly developed and the chloroplasts more numerous and larger. The chlorotic areas of the youngest leaves of *Eucharis amazonica* and *Amaryllis* affected with mosaic were thinner than the green ones and devoid of chloroplasts. In the third leaf both areas were thin, but the green one contained numerous intercellular spaces and more chloroplasts of normal dimensions. In the two older leaves also there were more chloroplasts in the green than in the chlorotic areas. Sheffield (48) states that as the virus of tomato aucuba mosaic does not affect the chloroplasts of leaves that are fully developed at the time of infection, it seems unlikely that mature plastids are destroyed by the virus. The mottling of young growing leaves, only, suggests that the virus prevents the formation of the plastids. In plants, infected with aucuba mosaic, certain of the leaf tissues are devoid of plastids and the cells may be undifferentiated. The absence of chlorophyll is brought about by the inhibition by the virus of the development of the plastid primordia. Usually the primordia are destroyed. If plastid development is not prevented in a very early stage, perfectly normal plastids are formed. Mature plastids are never affected by the virus, but occasionally intermediate stages may be.

It would seem, however, that although inhibited chloroplast development is a feature of certain virus chloroses, inhibition alone is not sufficient to account for the pathological effect in mosaic diseases of the potato. As Clinch has pointed out (12), the physical and chemical changes which may occur in the chloroplasts, the marked abnormalities in starch content in the chlorotic cells and the fatty degeneration which is also evident in them can scarcely be attributed to retarded development only. Clinch considers that there is evidence of definite physiological disturbances in the chlorotic cells, which may set in at a comparatively late stage of the development of the latter, acting in conjunction in many cases with an initial reduction in the size of the cells and a general under-development of their contents.

There seem, therefore, to be certain histological changes in the tissues which are common to those mosaic diseases causing a chlorosis without necrosis. First, there is a difference in lamina thickness between the dark green and chlorotic areas of the leaves, the dark green being as a rule thicker. This appears to be due to the inhibited development of the cells in the pale areas. Exceptions are found in corn mosaic and sugar cane mosaic, however, where the light green or chlorotic areas are thicker than the dark green areas. Secondly, as already stated, the chloroplasts tend to be reduced in number and size, while in acute infections they may break up into small hyaline granules. There is also a tendency towards starch accumulation. In abnormally dark green areas in mosaic-affected leaves, the cells are larger than normal and contain more chloroplasts and the chlorophyll itself is darker in colour (Dickson (13)). Here, then, the virus appears to have a stimulatory rather than inhibitory effect upon the development of the chloroplasts.

Another type of histological change is the necrosis due to viruses with a destructive action on the cells. The necrosed cells, especially sieve tubes and companion cells, collapse, and those surrounding them are stretched to a corresponding extent. Yellow discolorations may develop in the necrotic tissues which are thought to be attributable not to lignification but to suberization, *i.e.*, the infiltration of fatty acids. The necrotic changes caused by viruses in plant tissues have been chiefly studied in the potato plant and these will be discussed in some detail. Quanjer has described and classified five types of necrosis due to potato viruses. These are (1) *phloem necrosis*, this is restricted to the phloem and is characteristic of the disease known as leaf roll; (2) *Top necrosis*, radiating from a small percentage only of the internal phloem and seldom from the external phloem; (3) *Acropetal necrosis*, chiefly in the collenchyma of the leaf veins, petioles and stems; (4) *Pseudo-net necrosis;* (5) *Concentric necrosis.* These last two types of necrosis are confined to the potato tuber and occur in the storage parenchyma (Phytopath., 1931).

A necrosis of the potato tuber, known as "net necrosis," has been described (15). This consists of the appearance of brown strands in the vascular region of the tuber, involving the sieve tubes, companion cells and adjacent cells of the phloem parenchyma. Net necrosis is considered to be the first-season symptom of potato leaf roll and has only been described in America.

In a recent paper Bawden (3) describes three types of necrosis of the tissue of potato plants due to virus infections—namely (1) *Acronecrosis* (*top-necrosis*), where internal symptoms are produced in the petioles, stems and tubers, and consist of necrotic changes which originate in the phloem and spread thence to all other tissues; (2) *Acropetal necrosis* (leaf-drop streak) occurs on the stems and petioles affecting chiefly the collenchyma, the vascular tissue being normal; (3) *Phloem necrosis* (leaf roll)—here the necroses are confined to the phloem elements and consist of lignification.

Concerning the rolling of the leaves of potato plants affected with leaf roll, Quanjer (40) considers that this is due to the necrosis of the phloem, but, as Murphy (35) and Bawden (3) have pointed out, the necrosis is secondary to the rolling, as the latter may be very pronounced in the first season when the former is absent or very slight. A characteristic of the leaf-roll disease is the accumulation of starch in the leaves. In a young plant not yet showing the characteristic rolling of the leaves the chloroplasts appear normal, but with increasing development of the plant the starch accumulates and the chloroplasts become so full of starch that they may burst. This is the condition in the brownish edges of the brittle rolled leaves. The parenchymatous cells of the mid-rib of the leaf contain large storage starch grains, which are also present in greater quantity in the ground tissue of petiole and stem (12).

Effect of Environment on the Symptom Picture

The symptom expression of virus-diseased plants can be profoundly influenced by variations in the environmental conditions, the more important factors being temperature, light and humidity.

The effect of temperatures upon the clinical picture will be considered first. With certain exceptions it will be found that exposure to high air-temperatures tends to obscure or mask the symptoms of disease. This is especially true of the potato mosaic group and also of mosaic of tobacco. For example, if the growth of the host be taken into consideration, the optimum temperature for potato mosaic lies between 14°–18° C. Above 20° C. symptoms disappear, but a temperature of 24°–25° C. is necessary to cause them to disappear in one to two weeks, so that this temperature may be regarded as the maximum for mosaic manifestation in the potato (25).

As regards tobacco mosaic, air temperature has a marked effect upon the length of the incubation period of the virus in the plant, and this is probably true for the majority of viruses affecting Solanaceous plants. The optimum temperature for the activity of tobacco mosaic lies between 28°–30° C. with a maximum of about 36° C. When severely affected plants are placed at a temperature of 36°–37° C. for about a fortnight, the newly developing leaves are free of mosaic symptoms, while the older leaves regain a more or less normal appearance. If the plants are subjected once more to a lower temperature the symptoms reappear, showing that the virus is still active.

Temperature has a very decided influence in masking symptoms of two virus diseases of the raspberry, *i.e.*, mild mosaic and red-raspberry mosaic. At 28° C. plants affected with mild mosaic show no symptoms of mottling, and at the same temperature only very obscure symptoms appear on plants affected with red-raspberry mosaic. The yellow mosaic of raspberry appears to have a relatively higher temperature maximum for expression of symptoms as plants with this disease show distinct symptoms throughout the summer under Michigan conditions. Another virus disease, known as raspberry curl, shows marked symptoms at all temperatures at which affected plants will grow (5).

Symptoms of yellow mosaic of raspberry are modified but not masked by summer temperatures prevailing in Michigan (6).

There seems little doubt but that air temperature is the most important factor concerned in the masking or suppression of foliage symptoms. The effect of soil temperature, as Johnson has pointed out, appears to be largely a matter of growth response in the host, though it may increase the severity of the tuber symptoms in certain potato virus diseases.

It is possible to induce masking of symptoms of potato mosaic by comparatively short exposures to higher temperatures—thus mosaic plants placed for nine hours at temperatures above 24° C., but remaining at temperatures favourable to mosaic for fifteen hours, recovered in seven days. Plants exposed for only two hours at 28° C., but left at favourable temperatures to mosaic for twenty-two hours, practically recovered in twenty-eight days. At 40° C. two-hour and six-hour exposures led to recovery in seven and four days respectively (57).

It is worth recording in this connection that the condition of virus-infected potato plants known as " rust," which frequently occurs in the field, has never been observed in six years' study of virus-affected potato plants under glass. It seems probable that this condition is influenced by a combination of environmental factors, of which light intensity may possibly be one. Similarly, the characteristic " bronzing " of tomato plants affected with the virus of spotted wilt is much more pronounced out-of-doors than in the glasshouse.

The foregoing cases deal with the suppression of symptoms by high temperatures. There are certain virus diseases, however, which tend to develop more severely with rise of temperature. It has been observed in the case of mosaic disease of crucifers (see p. 329) that at temperatures of 70°–80° F. mustard and Chinese cabbage develop a severe streak and other plants are much stunted. At 55°–65° F., however, even the more susceptible hosts make a fair development, while mosaic brussels sprouts and cauliflower often cease to show mosaic symptoms and grow normally. Similarly, in "yellow dwarf" of potatoes, a virus disease not recorded in Europe, very severe symptoms develop at 25° C., while no symptoms appear at 15° C. (17). Curly top of sugar beet also develops more severe symptoms at high than at low temperatures.

As regards the effect of light upon the clinical picture, Price (39) states that tobacco plants, inoculated with the virus of a ringspot disease, develop water-soaked necrotic spots when kept in the darkness, and are eventually killed by the disease instead of recovering, as under normal conditions. In certain cases the effect of shading, as distinct from darkness, seems on the other hand to be beneficial to the virus-affected plant. Shapovalov and Lesley (46), who have studied in some detail the effect of shading upon the rate of development of tomato "yellows" (= the virus of curly top of sugar beet) find that shading increases the tolerance of the plants to the virus. If continued after the occurrence of infection, shading enables the plants to produce a crop, and even facilitates complete recovery in some cases (see p. 241). This is in accordance with the observations of Carter (7), who found that high light-intensity associated with high temperatures favoured the development of severe symptoms in the case of the same virus on sugar beet. It has also been shown (45) that four to six hours' exposure to four 500-watt Mazda lamps accelerated the rate of disease development of tomato yellows while shading retarded it. Another instance of the beneficial effect of shading is given by the infectious chlorosis of Abutilon where affected plants, grown in the shade produce leaves of normal green, and even appear to recover altogether in some cases.

In the case of the viruses of certain tomato and tobacco mosaics which produce leaf abnormalities in the tomato plant, it is considered that under longer-day conditions mottling may be the only effect after the primary bunching of the young leaves is seen. The production of leaf abnormalities appears to be correlated to a greater degree with low-light intensity than with short-day length. Under conditions of low-light intensity the virus of cucumber mosaic produces on tomato only mottling without leaf deformity (37), (cf. Mogendorff, p. 142).

In considering the symptom picture induced in plants by viruses, the outstanding fact is the immense range of symptoms which one virus entity is capable of producing. This symptom range is governed by two main factors, the species of host plant and the environmental conditions under which the affected plants are grown. It is the variability in the symptom expression which makes differentiation of viruses by this means so difficult and which is partly responsible for the confusion at present existing in the study of plant viruses in general and of the potato and tobacco mosaic groups in particular.

An interesting point which does not seem to have been investigated is the question as to whether masking of mosaic symptoms by high temperatures involves the disappearance of the intra-cellular inclusions. To this may be added the question as to whether X-bodies are ever found in a potato plant which is " carrying " without symptoms a mosaic virus with which X-bodies are associated in other non-carrying potato varieties. In other words, if the production of X-bodies could be definitely correlated with the production of mosaic mottling, this would seem to be further evidence of the non-parasitic nature of these cell-inclusions.

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CHAPTER III

PHYSICAL PROPERTIES

Reactions of viruses to heat, chemicals and dilution—Longevity in vitro—*Effect of light.*

THE study of the physical properties of viruses is of importance as being perhaps the most likely avenue of approach to a realization of their true nature, and the co-operation of the physical chemist and bio-chemist with the biologist in this matter is greatly needed.

The only evidence that a plant virus exists at all is the pathological effect which it produces upon its host, and it is chiefly owing to this fact that symptoms have acquired a somewhat exaggerated importance in the study of the subject. There is a very great need at the moment for some reliable method of classifying and differentiating plant viruses, and any aspect of the study which is likely to further that aim is for that reason alone worthy of careful consideration. A classification of plant viruses based upon their physical properties has been advocated by James Johnson (63), who has attempted to classify by this means the viruses affecting the tobacco and potato plants. There are, however, two serious difficulties in the way of a successful application of physical tests to virus differentiation. The first of these, which is common to both plant and animal viruses, is the wide range in the degree of resistance to physical and chemical agents which renders difficult any classification based upon resistance to such agents. The second difficulty, which is not encountered by the workers with animal viruses, is the existence of a large group of plant viruses which are not sap-transmissible but are only insect-borne. This second difficulty may, it is true, be partially overcome by subsequent improvements in the technique of feeding insects upon virus solutions (see Chapter V., p. 126), but it is likely to be a considerable time before the physical properties of viruses which are not sap-transmissible can be thoroughly tested by these means.

Reactions of Viruses to Heat

All viruses, whether affecting plants or animals, are destroyed at relatively low temperatures, but the thermal death-points show a very wide range of variation. As regards plant viruses, at one end of the scale is the tobacco mosaic virus with a thermal death-point between 80° and 90° C., while at the other end are found the viruses of tomato spotted wilt and cucumber mosaic, which are inactivated at temperatures of 42° C. and 43° C. respectively. McKinney describes (83) a convenient method of measuring thermal inactivation. Small quantities of virus extract, contained in thin-walled glass tubes, 45 mm. long by 7 mm. inside diameter, closed at each end by a tight-fitting cork and secured by a cord, are maintained wholly submerged at a constant temperature in an insulated water bath 6 in. deep and 8 in. in diameter. The bath is furnished with a standardized thermometer placed at the level of a wire tray, which serves both to secure the tubes and to act as an agitator. He finds that a hand-regulated gas flame is sufficient to control the temperature to within 0·2° C., and that fifteen seconds should be allowed for the tubes and their contents to reach the temperature of the bath. At the close of the experiment, tray and tubes are removed together and plunged at once into cold water.

The virus of tobacco mosaic has been very extensively studied, and the effect of heat upon this virus will be considered first. It must be realized, however, that there almost certainly exist a number of distinct viruses which cause tobacco "mosaic," and it is probable that not all the workers on this subject are concerned with the same virus entity. This difficulty arises from the lack of a stable means of virus differentiation and its effect on plant virus studies must be recognized. Allard (1) found that the virus of tobacco mosaic was quickly killed at temperatures between 80° and 90° C. In dried and ground mosaic material, rendered water-free by drying over H_2SO_4 in a desiccator, the virus appeared to resist higher temperatures than when in solutions. Mulvania (88) gives the thermal death-point at 90° C.

James Johnson has studied the effect of heat on nine tobacco viruses (63); of these, however, only five or six are apparently of the same type as those studied by Allard and Mulvania. The thermal death-points of these six viruses are as follows :—

Tobacco mosaic (tobacco virus 1)	90° C. for ten minutes.
Speckled tobacco mosaic (t.v. 2)	90° C. ,, ,, ,,
Mild tobacco mosaic (t.v. 3)	60° C. .. .. ..
Yellow tobacco mosaic (t.v. 6)	90° C. ,, ,, ,,
Medium tobacco mosaic (t.v. 7)	90° C. ,, ,, ,,
Bleaching tobacco mosaic (t.v. 8)	75° C. ,, ,, ,,

It will be seen that four out of these six viruses have the same thermal death-point of 90° C. It has been shown by the same worker (62) that exposure to temperatures of 35°–37° C. for ten days attenuates the virus of tobacco mosaic. In a recent paper examining the properties of tobacco mosaic (tobacco virus 1) for a number of different plant species, Johnson and Grant (65) find the thermal death-point of this virus to be slightly below 90° C. Studies in England (113) upon the aucuba mosaic of tomato, which is thought to be the same virus as the yellow tobacco mosaic or tobacco virus 6 of Johnson, indicate that this virus will withstand heating for ten minutes at 80° C., but is inactivated at 90° C. Another tomato virus of the tobacco mosaic type, which is known in England as "stripe," and may be the same as Johnson's tobacco virus 9, is also inactivated at 90° C. (15). "Typical" tomato mosaic is inactivated by short exposures at a temperature of 88° C. and by longer exposures at temperatures of 82° and 84° C. (21). McKinney (83) is of the opinion that the thermal death-point depends to some extent upon the concentration of the virus and puts it at 88°–90° C. in an undiluted state, and at 82°–84° C. for virus diluted 100 times. He also considers that the species of host plant affected may influence the thermal death-point of the virus. This point of view is no longer tenable (65). It will be seen that, with two exceptions, the viruses of the tobacco mosaic type are inactivated at about the same temperature, *i.e.*, 90° C., and this is the highest thermal death-point at present recorded for plant viruses. These various tobacco mosaic viruses have other properties in common, such as extreme ease of transmission by inoculation, longevity *in vitro*, filterability, etc., and are probably closely related (see Chapter XIV., p. 368).

The available information on the physical properties of potato viruses is not large, and this is due partly to the confused state of the knowledge of this group of viruses and partly to the fact that several of the viruses are not sap-transmissible, as, for example, those of potato leaf roll, paracrinkle (108) and the " A " virus of Murphy and M'Kay (89). The following are the thermal death-points of certain potato viruses (64): 43° C. for crinkle mosaic; 40°–45° C. for mild mosaic; 60°–65° C. for rugose mosaic; and 70° C. for the mottle form of rugose mosaic. Henderson Smith (114), working with mosaic from various potato varieties, gives the thermal death-points at 70°–80° C. It is probable, however, that in certain of the above cases the workers were sometimes dealing with mixed viruses. The writer's X potato virus is inactivated by exposure to a temperature of 66° C. for ten minutes, while the Y virus is inactivated by exposure to a temperature of 52° C. under similar conditions. Goss (50), working with potato spindle tuber and unmottled curly dwarf, obtained 30 per cent. infection after exposure at 75° C. for ten minutes, but no infection at 85° C.

The following are the thermal death-points of a number of other plant viruses, arranged in a descending order: tomato streak, above 80° C. (29); mosaic of dock *Rumex obtusifolius* and *R. lanceolatus*, 80° C. for ten minutes (51); yellow dwarf of onions, 75°–80° C. for ten minutes (55); tobacco ringspot, 60°–70° C. (54); sugar beet mosaic, 55°–66° C. for ten minutes (60); cucumber mosaic, 43° C. (39); spotted wilt of tomato, 42° C. for ten minutes (9). As regards curly top of sugar beet, which is not transmissible by inoculation, Carsner and Stahl (23) consider that the virus of this disease will stand temperatures not much below those at which the beet tissue is killed.

The thermal death-points of plant viruses, then, range from approximately 90° C. in the case of tobacco mosaic to 42° C. for the virus of tomato spotted wilt. This variation is closely paralleled in the animal virus group where the thermal death points range from 70° to 45° C. The following are the inactivation points of a few representative animal viruses: the activity of the tumour filtrate of Rous' sarcoma is destroyed at 55° C. for fifteen minutes (106); yellow fever, the virus of which is very susceptible to heat, is destroyed by exposure to 55° C. for five minutes (*in* 121 p. 449); foot-and-mouth disease, inactivation commences at 50° C. and increases at a uniform rate with each rise of temperature of 1° C. (*in* 121 p. 69); vaccinia, vaccine lymph freshly collected from the calf and mixed with water withstands a temperature of 57·5° C. for five minutes without loss of virulence, but exposure to 60° C. for the same time is lethal (18); fowl plague, 55° C. for one hour (66); dog distemper, thirty minutes at 60° C. (77); caterpillar wilt, 60° C. for twenty minutes.

The reactions of viruses to sterilization by heat do not appear to differ materially from the reactions to heat of ordinary bacteria.

As regards the effect of drying upon plant viruses, the virus of tobacco mosaic is very resistant to desiccation and will remain viable for periods of years in dried plant tissue. On the other hand, the virus of tomato spotted wilt, of cucumber mosaic and the X and Y potato viruses are inactivated by desiccation. The virus of dog distemper will withstand drying for a time, while that of Borna disease retains its virulence for at least 306 days in the dry state. On the other hand, the slightest desiccation is fatal to the virus of yellow fever (121).

According to Giddings *et al.* (48), some experiments on the effect of high pressure upon the virus of tobacco mosaic have proved that, while 75,000 lb. pressure had little effect, no infection developed in any plants inoculated with mosaic juice subjected to pressures of 130,000 lb. or over. The pressure required to inactivate tobacco mosaic virus is higher than the pressure death-point of a number of bacteria, but lower than that of *Bacillus subtilis*. The enzyme zymase is inactivated at a lower pressure than that required to destroy the tobacco mosaic virus, the pressure death-point of which probably corresponds approximately to that of pepsin. In a recent note to *Science*, Olitsky and Forsbeck (93) state that they have inactivated the virus of a mosaic upon tomato by pulverization of the tissues.

Chemicals

Concerning the resistance of viruses as a group to chemical agents, there is one point of behaviour in which they appear to differ from the reaction of the majority of bacteria, namely, in their resistance to the action of glycerol. The writer has found that the potato virus known as X will remain viable in 50 per cent. glycerine for twenty-two days. Klebahn (67) states that the virus of tobacco mosaic will resist glycerine for several days. There do not appear to be many recorded instances of the resistance of plant viruses to glycerine, and recourse must be made to the literature on the animal viruses for information on this point. Rivers (103) considers that the extensive use of glycerol for the preservation of viruses is probably largely dependent upon the fact that viruses are usually very susceptible to conditions in autolysing tissues, and that glycerol acts as a desiccant and retards autolysis of the tissues containing the viruses.

The effects of a number of miscellaneous chemical agents have been tested upon the virus of tobacco mosaic. Allard (2) found that ether, chloroform, carbon tetrachloride, toluene and acetone failed to destroy the infective principle, and the following also had little effect on the virus : nitric, hydrochloric, phosphoric, citric, acetic and carbolic acids, manganese sulphate, sodium chloride, aluminium sulphate, lithium nitrate, sodium nitrate, silver nitrate and mercuric chloride. On the other hand, the virus was quickly destroyed by 80 per cent. ethyl alcohol and 40 per cent. formalin The virus of tobacco mosaic appears to resist various kinds of alkaloid, salts of alkaloid, glucoside and ethereal oil when it is subjected to those chemicals in the mosaic tobacco juice. Oil of mustard in 2 per cent. and digitalin in 5 per cent. concentrations destroyed the virus in five days. Nicotine in 1–2 per cent. and atropine in 1 and 2 per cent. concentrations were toxic to the virus, while 2 per cent. saponin had no appreciable effect (45). Oxygen appears to inactivate the virus of tobacco mosaic when properly exposed to it under moist conditions (62). On the whole this virus appears to be relatively resistant to chemical and physical agents, and in respect to inactivation Duggar (31) considers it to be in a class parallel with enzymes, certain spores and some of the more resistant vegetative cells.

The effect of alcohol upon a number of viruses has been investigated; the virus of tomata aucuba mosaic (probably the same as Johnson's tobacco virus 6) withstands alcohol in strength up to 90 per cent. (113). Potato viruses are much less resistant to alcohol and other chemical agencies than viruses of the tobacco mosaic type. Thus, according to J. Johnson (64), 25 per cent. alcohol inactivates the viruses of crinkle and rugose mosaic in one hour. Henderson Smith (114), using mosaic viruses from different potato varieties, found that inactivation of these was brought about by alcohols of between 80 and 90 per cent. strength. The virus from potatoes—Majestic and Arran Chief—showed higher resistance than that from potatoes Up-to-Date. The writer has found that the potato viruses X and Y are inactivated by 75 per cent. alcohol. The virus of bean mosaic is sensitive to alcohol, being inactivated by exposure to 25 per cent. for thirty minutes (39), while the virus of dock mosaic will withstand exposure to 95 per cent. for thirty minutes (51).

The following are the reactions of some representative plant viruses to various other chemical agents: the potato mosaic group is sensitive to nitric acid and formaldehyde. Nitric acid (1 : 500) inactivates the viruses of crinkle and rugose mosaics, while in tobacco the potato rugose virus is destroyed by 1 : 200 formaldehyde, but the mottle form of this disease is only destroyed by 1 : 100 formaldehyde (64). Rugose mosaic of potato is now known to consist of a complex of viruses (71). Formaldehyde at dilutions of 1 : 500 (but not at 1 : 1,500), acting for two hours at 27° C., destroys potato mosaic from varieties Majestic and Arran Chief. The same virus in filtered juice withstands " 20 per cent. chloroform " at 27° C. for four hours and the dyes Auramine O and

Meldola Blue, diluted 1 : 2,000 for two hours at 27° C., while Acriflavin (1 : 1,000) does not wholly destroy it (114).

Certain plant juices have an inactivating effect upon viruses in some cases ; thus it has been demonstrated that the sap of poke-weed, *Phytolacca decandra*, inactivates the virus of tobacco mosaic at a dilution of 1 : 5. The juice of *Datura stramonium* was also effective at a relatively high dilution (1 : 100) (32). At the same time it must be pointed out that *Datura stramonium* is susceptible to, and frequently killed by, more than one virus of the tobacco mosaic type. The writer has tested the effect of turnip juice upon the potato virus X in 50 per cent. dilutions in an attempt to investigate the possible effect on this virus of the bactericidal substance " lysozyme," described by Fleming (42) as being present in this plant. The following tests were carried out : in the first, untouched turnip juice was used ; in the second the juice was boiled before mixing with the virus juice ; and in the third test the juice was filtered through an L_3 Pasteur-Chamberland candle before being mixed with the virus. All three solutions were allowed to stand overnight before inoculation to the test plants. In every case a 100 per cent. infection was obtained, showing that the turnip juice had not affected the virus. Further tests of this nature are being carried out, using egg albumen in place of turnip juice. Some other experiments on the effect of true enzymes upon plant viruses have been made by Lojkin and Vinson (80). Under the conditions of the experiments, incubating the virus preparations with emulsin, pepsin or yeast extract did not reduce the infectivity. Trypsin, pancreatin and papain inactivated the virus ; erepsin inactivated the virus only after an incubation period of several days. None of the enzyme preparations had any effect on the virus in fresh untreated juice, and the inactivating effect of the enzymes was destroyed by boiling.

In experiments with trypsin and pepsin digestion of a suspension of the virus of tomato aucuba mosaic, while pepsin appeared to have no effect on the number of local lesions on *Nicotiana glutinosa*, trypsin had the effect of reducing the number. That this was not due to a definite destruction of the virus agent by the enzyme is indicated by the fact that heating the virus material to 70° C. for twenty minutes after incubation has the effect of increasing the number of spots, on subsequent inoculation, to a number comparable with that formed by the control material. Both taka-diastase and malt diastase, freshly prepared from barley, had a similar effect to that of trypsin in reducing the number of spots which were formed, and heating in these cases did not restore the efficiency of the inoculum. It was not possible, however, to destroy those enzymes, as judged by their effect on starch, without heating above 70° C. This would, at the same time, have destroyed the virus which will withstand heating to 80° C. for only a short period. At the same time, boiling the enzyme in water before treatment of the virus had the effect of rendering it innocuous (22).

The following reactions of a few representative animal viruses to chemical agents are given for comparison with the foregoing. The virus of the Rous fowl sarcoma is destroyed by toluol, chloroform, 50 per cent. alcohol and 2 per cent. phenol (106). The virus of foot-and-mouth disease is very resistant to chloroform and ether (121) and is fairly resistant to alcohol. Formaldehyde destroys this virus easily, while various aniline dyes are weakly destructive to it. Phenol has a special importance as an antiseptic against the virus of rabies. The virus of cattle plague was found to be inactivated by treatment for three and a half hours at 37° C. with 10 per cent. chloroform (26). The virus of fowl-pox has been exposed to various chemicals. In an emulsion of scabs it resisted 1 per cent. phenol water for one and a half hours, 2 per cent. inactivated it in thirty minutes. It is inactivated in five minutes by solutions of 1 per cent. KOH, 1 per cent. acetic acid and bichloride of mercury 1 : 1,000.

The resistance of animal viruses generally to various chemical agents appears to be of the same general order as that exhibited by plant viruses.

In all experimental work relating to the physical properties of viruses it is well to bear in mind that it is not certain whether a virus, either plant or animal, has yet been obtained in a pure state, and the presence of proteins may affect very considerably the apparent reactions of the virus. The important question of the purification of virus suspensions is dealt with in more detail on p. 65. Kligler and Olitski (68) present data which show that the effect of heat, and more particularly that of various chemicals, on a bacteriophage and on fowl-pox virus is weakened by the presence of protein. There is a general similarity in the reactions of the pure fowl-pox virus and bacteriophage to disinfectants, but there are differences in their relative susceptibility to one or another antiseptic.

Dilution

In considering the dilution end-points of plant viruses, it will be noticed that as wide a variation exists as is the case with other properties of those agents. At one end of the scale will be found the virus of a tobacco mosaic with a dilution end-point of 1 : 1,000,000, and at the other end the virus of dock mosaic and certain potato viruses which begin to show inactivation at dilutions of 1 : 100 or even less. The virus of aucuba mosaic of tomato when diluted to 1 : 10,000 gives a 37·5 per cent. infection, but no infection at greater dilutions (113). Potato mosaic viruses are less tolerant of dilution than those of the tobacco mosaic type. James Johnson (64) considers that a dilution greater than 1 :10 usually results in rapid diminution of the percentage of infection. Crinkle mosaic and mild mosaic are apparently the most readily inactivated, withstanding with difficulty dilutions between 1 : 10 and 1 : 100. Leaf-rolling mosaic may still be quite infectious at 1 : 200, while rugose mosaic, now known to consist of more than one virus, apparently will not stand a dilution of 1 : 1,000. Henderson Smith (114), using mosaic from various potato varieties, found infection up to dilutions of 1 : 1,000. The writer, experimenting with potato viruses X and Y, finds that X will stand dilution of 1 : 10,000 and rarely gives infection at 1 : 100,000 ; while Y begins to be inactivated at 1 : 100, and only occasionally gives any infection at 1 : 1,000.

The following are the dilution end-points of a number of other viruses. Potato spindle-tuber, 50 per cent. infection at 1 : 10^3 ; unmottled curly dwarf, 10 per cent. infection at 1 : 10^3 (50) ;

tobacco ringspot, infectious at dilutions of $1 : 10^3$, but only a trace of infection at $1 : 10^4$ (54); bean mosaic gives no infection at dilutions higher than $1 : 10^3$ (39); dock mosaic gives positive infections at $1 : 10^2$ but not at $1 : 10^3$; cucumber mosaic exhibits a marked falling off in infective power at $1 : 10^4$; sugar beet mosaic is inactivated at dilutions above $1 : 10^3$ (60).

Animal viruses appear capable of withstanding considerably higher dilutions than do viruses affecting plants. As regards foot-and-mouth disease, the British and American Commissions have found the limit of infectiousness of the virus to be at a dilution of 1 in 10 million. Similarly, the virus of polyhedral disease of caterpillars is infectious at a dilution of 1 in one million. The degree of dilution which herpes virus will stand without loss of activity varies with different strains. Rabbits' brain strains show titres, from 1 : 1,000 to 1 : 1,000,000, while skin strains are rarely active beyond a dilution of 1 : 10,000 (121 p. 160).

There are two suggestions which may be offered to account for the inactivation of viruses by dilution. The first of these explains the loss of infective power upon purely mechanical grounds by the dispersal of the virus particles. On this assumption positive infections would be expected to occur occasionally at very high dilutions. The second suggestion is that a definite inactivation results, which is due to the dissociation of the virus particles from their colloidal support. It is clear, then, that if the first suggestion is the correct one, the inactivation of the virus would be reversible, while in the second case, presumably, it would be irreversible. There do not appear to be any references in the literature to experiments which might confirm those points. Using Elford's membranes (see p. 75), the writer has attempted to reactivate the potato virus X in dilutions of 1 : 100,000, at which dilution the virus is not active, by concentrating the solution on a membrane which is impermeable to the X virus. These experiments are still in progress.

Longevity in Vitro

The length of time that plant viruses will remain viable in sap extracted from an affected plant and cleared of cells and visible particles varies over a wide range among the different viruses. Thus the virus of tobacco mosaic is known to persist in an active state in filtered sterile juice for a period of years, while the longevity in extracted sap of the viruses of tomato spotted wilt, cucumber mosaic and the Y virus of potato is measured in periods of hours only. Holmes (61) stored the virus of tobacco mosaic under different temperature conditions and found that virus kept at freezing point lost strength less than samples kept in a bottle at room temperature. He concludes that a virus does not retain all of its strength over long periods of time. As regards other viruses of the tobacco mosaic type, filtered juice of tomato aucuba mosaic was found to be still active after being stored in subdued light at room temperature for a year or more (113). A form of tomato streak remains viable for at least 108 days (29), and the writer has worked with the virus of a tomato mosaic which was still viable after several months (115). Certain other viruses lose their infective power very rapidly when outside the plant host. The virus of cucumber mosaic becomes inactivated in twenty-four to forty-eight hours and is never infectious after three days' ageing (65), while the virus of sugar beet mosaic remains viable for twenty-four to forty-eight hours at about 70° F. (60). The virus of " yellow dwarf " of onions is inactivated after 112 hours in sterile water at 29° C. (55), while the virus of spotted wilt becomes inactivated after four to six hours' ageing (9, 115).

As regards potato viruses, the writer, working with the two entities known as X and Y, has found that X remains viable in filtered sterile juice for seventy-eight days, while Y is inactivated after twenty-four to forty-eight hours' ageing, at room temperature, in juice clarified by passage of a sand-and-pulp filter or permeable collodion membrane. Goss (50) considers that the viruses of potato spindle tuber and unmottled curly dwarf are inactivated rapidly after being extracted from the plant.

There are several records indicating extreme longevity of certain viruses in dried tissue. In tobacco mosaic the active principle was found to be still viable in leaves that had been dried and preserved for twenty-four years. Dickson (27) offers evidence that the same type of virus was still active after five years in dried leaves, and Johnson (62) finds that tobacco mosaic virus may retain its viability for several months in the soil and considers that its duration of life is much shorter in sandy than in clay soils. This last fact may be determined by the oxygen relations. According to Pierce and Hungerford (97) the virus of bean mosaic is capable of remaining viable in the seed for a period of thirty years.

Similar studies on viruses affecting animals show that the virus of foot-and-mouth disease remains infective in hay or garden soil under field conditions for twenty-five to thirty days (109), while material from fowl-pox lesions, dried and kept at room temperatures for two years, has proved to be infectious. The virus from silkworms affected with grasserie is capable of remaining infective in blood, dried at room temperature, for at least two years (121 p. 58).

It is not known whether the rapid inactivation of some plant viruses in extracted sap may not be due to certain changes, such as oxidation, taking place within the plant juices containing the virus. It is possible that certain viruses are more susceptible to oxidation than others, and this may be one reason for the extremely short life, in extracted sap, of the viruses of tomato spotted wilt, cucumber mosaic and the Y virus.

The writer has found that the longevity *in vitro* of the potato virus Y in extracted sap at ordinary laboratory temperatures lies between twenty-four to forty-eight hours, but if the sap is kept at — 10° C. the virus remains viable for as long a period as 141 days. Temperatures such as + 1° C., while prolonging the active period of the virus, do not have the same effect as the low temperature. It seems possible that this rapid inactivation of the Y virus in extracted sap may be due to oxidative processes and that such oxidation is arrested when the sap is stored at — 10° C. It has also been found that additions of small quantities of carbolic acid to the expressed juice increases the resistance to ageing of a tobacco ringspot virus. Virus-infective juice to which sufficient 5 per cent. carbolic acid had been added to make a concentration of 0·25 per cent. was highly infectious after incubating for seven days at 27° C. The virus in juice treated with toluene was not infectious on the seventh day and in untreated juice on the third day (53).

As regards animal viruses: the inactivation of the virus of Rous' sarcoma in filtrate during incubation is also thought to be due to oxidative changes. From a study of the effect of various inhibitors of oxidation and proteolysis on the activity of the Rous' sarcoma filtrate under different conditions, it was concluded that the inactivation of the filtrate which occurs at 37° C. is due to oxidation. It has been shown that partial reactivation of the filtrate may occur in the presence of reducing agents, and that sodium pyrophosphate and the hydrogen-palladium reducing system preserve the activity of the filtrate for at least twenty-four hours (99). Gye and Purdy (52) have shown that although the filtrate from a Rous' sarcoma became inactive if it were incubated at 37° C. for twenty-four hours, the addition of small amounts of hydrogen cyanide caused the filtrate to retain its activity at 37° C. for as long as three days.

Some account of studies on the influence of oxidation and reduction on the virulence of herpes virus is relevant here (131). It seems a likely conjecture that the attenuation of herpes virus on keeping is due to oxidation, and that subsequent " resuscitation " can be induced by the reduction ensuing on addition of cysteine (cysteine hydrochloride: 2 per cent. cysteine solution freshly made to a final concentration of 0·1 per cent.). Filtrates sealed immediately without cysteine will remain potent much longer than those in open tubes; and those sealed immediately with cysteine will retain their activity for three weeks or longer at room temperature; both these facts are indications that free access of oxygen is concerned in the deterioration. It has also been found that herpes filtrates kept in open tubes, either with or without glycerine, in the ice box retain viability for five to six weeks, but if kept at room temperature inactivation ensues at the end of two to four days, and in the incubator degeneration is correspondingly more rapid (cf. potato virus Y above). Cysteinized and under a seal, similar filtrates would remain active at room temperatures for three weeks or longer.

Perdrau and Todd (96) have shown that the viruses of bacteriophage, vaccinia, herpes, fowl-plague, louping-ill, Borna disease and canine distemper, as they exist in filtrates, are highly sensitive to the photodynamic action of methylene blue—a concentration of 1 part in 100,000 inactivating the viruses within a few minutes under suitable illumination. This inactivation appears to be an oxidative process, as in experiments with the viruses of herpes and vaccinia no inactivation took place in the absence of free oxygen.

Effect of Light

It is the effect of light upon the virus itself which is considered here, and not the effect upon the disease, which is dealt with in Chapter II, p. 43. Dickson (27) has carried out a number of experiments on the effect of light on the virus of tobacco mosaic. He tested the effect of red, green and blue light, sunlight and darkness upon the extracted sap of diseased tobacco plants. Dickson considered that there was an attenuation of the virus in the vials kept under blue, red and green light in descending order as given, but found little difference between the red and the blue. Darkness and green light were approximately similar in their effect.

As regards the effect of ultra-violet light upon plant viruses, not much investigation has been carried out in this direction. According to Mulvania (88) who exposed the extracted juice of mosaic tobacco plants to ultra-violet light for periods of one-sixth to four hours, exposure of one hour or more inactivated the virus. Similar experiments by Fanny F. Smith (112) indicated that the shorter or abiotic ultra-violet rays inactivated tobacco mosaic in filtered juice after thirty minutes' exposure, while under similar conditions a suspension of *Bacillus prodigiosus* was killed in thirty seconds. Smith considers that the long period of irradiation which was apparently necessary for inactivation of the virus to take place is evidence against the theory that the causal agent of a plant virus is of a biological nature. These findings of the above two workers are not borne out by later experiments by Arthur (5) and Arthur and Newell (6). The former, experimenting with radiation from a mercury vapour arc in quartz, found, when using appropriate filters, that injury to the plant tissue begins at a wavelength shorter than 285 $\mu\mu$ and increases rapidly with decreasing wavelength, the killing time of direct exposure of virus without filters being less than fifteen seconds. Further experiments in this direction (6) show that the virus of tobacco mosaic is completely inactivated by fifteen seconds' exposure to the open arc, provided that the virus is sufficiently free from contaminating material. These workers consider that a virus, whatever its nature, can be inactivated by extremely short exposures to ultra-violet radiation so long as it is sufficiently free from impurities. In this connection, a statement of Galloway and Eidinow (46) is relevant. In investigating the effect of ultra-violet light on the viability of the virus of foot-and-mouth disease, they state that when the virus was suspended in *unfiltered* lymph or serum, a protective action due to adsorption by the suspending fluid was observed. In this connection it is of interest to note that Perdrau and Todd (96) found that protection was afforded against the photo-dynamic action of methylene blue on certain viruses when in the presence of living cells. In the case of a bacteriophage it was found that the presence of living bacteria—not necessarily of a specific bacterium—greatly increased the resistance of the bacteriophage to the photo-dynamic action of methylene blue. Further experiments by Arthur and Newell showed that the virus of tobacco mosaic could be killed by short exposure (one minute) when spread upon the leaf surface. If irradiated the day following inoculation, there was no appreciable killing of the virus. It was not found possible to inactivate the virus after it had penetrated into the plant tissue, even if irradiation were given of sufficient intensity to kill the whole upper surface of the leaves.

A considerable amount of work has been carried out upon the effect of ultra-violet light on animal viruses and some results of this work are quoted here for comparison with the foregoing experiments. Susceptibility of Rous' fowl sarcoma virus to ultra-violet rays has been definitely shown, short exposures completely destroying the infectivity of the tumour filtrates. In this case the dose of irradiation necessary to cause complete inactivation is considerably in excess of that required to sterilize dilute bacillary emulsions (8). The virus of foot-and-mouth disease in filtrates is destroyed after five minutes' exposure to the radiations of the mercury vapour lamp in quartz flasks, which transmit radiations of wavelengths from 5,720 to 2,300 Å.U. It is inactivated after thirty minutes' exposure to the rays of the mercury vapour lamp filtered through a sheet of Vita glass, which allows passage of rays from 5,720 to 2,800 Å.U. The rays of wavelengths 5,700 to 3,300 Å.U. have no lethal action on the virus of foot-and-mouth disease. The susceptibility of a bacteriophage to ultra-violet irradiation is of the same order as that of bacteria and the relative lethal efficiencies of different regions of the spectrum are the same for a bacteriophage as for *B. coli* (7). The work of Rivers and Gates (105) indicates that the susceptibility of vaccinia virus to irradiation is also of the same order as that of bacteria.

CHAPTER IV

PHYSICAL PROPERTIES (*Continued*)

Purification of virus suspensions—Filtration and adsorption—Size of virus particles—Particulate nature—Electric charge of virus particles.

Purification of Virus Suspensions

An important question in dealing with the physical properties of viruses is that of purification of virus suspensions, particularly the production of protein-free suspensions of the virus. It has already been indicated that viruses may be protected from the influence of ultra-violet light if irradiated in impure solutions, and this masking effect of associated proteins may apply also in investigations on other properties, such as electrical charge, resistance to heat and chemicals, filterability and so on.

The following are the chief methods of purification of virus suspensions: precipitation by various chemicals, selective adsorption and eleution, centrifuging, temperature coagulation and concentration on collodion membranes.

Precipitation. Alcohol of different strengths, acetone and calcium chloride have been used for the precipitation of certain plant viruses in solutions. Thus the virus of " aucuba " mosaic of tomato in filtered juice is precipitated by 60 per cent. alcohol, and the supernatant fluid appears to be inactive after filtration (113). The virus of a tobacco ringspot can be precipitated and separated from expressed juice with either alcohol or acetone and then recovered in water without any appreciable injury to its infective properties (54). Duggar (31) has shown that calcium chloride when added to mosaic tobacco juice produced a precipitate which carried down the virus.

A considerable amount of work upon the precipitation of the virus of tobacco mosaic in suspensions has been carried out by Vinson and Petrie. In their first published work (127), an aqueous solution of safranin was used to precipitate the virus from the juice of mosaic-diseased tobacco plants. The precipitate thus produced is said to contain practically all of the virus but to retain it in an inactive condition. The virus can be released when the safranin is removed by means of amyl alcohol. Ammonium sulphate and magnesium sulphate were also used to salt out from infective juice, material which when redissolved in water was found to be infectious.

Acetone or alcohol, in the proportions of two volumes to one of juice from diseased plants, throws down a precipitate at about 0° C., which is said to contain practically all the virus. Low concentrations of lead acetate and barium acetate have also been used to remove some of the phosphate, sulphate and most of the protein and pigment from infective juices. Vinson and Petrie consider that the virus of tobacco mosaic behaves in many ways in a manner analogous to a chemical substance. In the foregoing experiments the virus of tobacco mosaic is thought to be freed from 90 per cent. of the accompanying solids.

In a further paper Vinson and Petrie (128) state that the total solids of the virus fraction have been reduced to about 1 per cent. of that of the original material without any reduction in infective power. Owing to the difficulty of obtaining a precipitate free from brown pigment with the safranin method of precipitation, a solution of lead acetate was used to precipitate the virus. The lead precipitate of the virus is then suspended in a normal solution of primary potassium orthophosphate, which is stated to remove irrelevant organic material but not much of the virus. The latter is then eleuted by suspending the precipitate in an M/15 solution of potassium phosphate with a hydrogen-ion concentration of about *p*H 6·5.

In a more recent publication Vinson (126) states that progressively increasing the amount of a safranin solution added to a given amount of juice from diseased plants results in a progressive decrease in concentration of virus remaining in the supernatant liquid. The virus may be re-precipitated with safranin without appreciable loss of virus being demonstrable. When two volumes of acetone are added in the cold to one volume of the virus fraction obtained on decomposing the safranin precipitate, a precipitate forms, but considerable virus seems to remain in the supernatant liquid.

If the results of these precipitation methods are confirmed by other workers, and if they are applicable to plant viruses other than that of tobacco mosaic, it is evident that such purification of virus suspensions will be of great value in filtration and other studies.

Selective Adsorption and Eleution. It may be possible to make use of the capacity for adsorption possessed by viruses for producing protein-free suspensions. The writer has shown that of two potato viruses, known as X and Y, one, Y, at a *p*H of 5·9, will adsorb to a kieselguhr or kaolin bed while the other, X, passes through and is found in " pure culture " in the filtrate. This capacity for adsorption on the part of the virus Y may be made use of to obtain a purified virus suspension, and experiments to this end are now being carried out.

Somewhat similar methods of purification to the foregoing have been used in obtaining purified suspensions of animal viruses. Kligler and Olitski (68, 69) have carried out studies on protein-free suspensions of a bacteriophage and of fowl-pox virus. Kaolin was used as the adsorbent and 1 c.c. of bacteriophage broth was added to 1 c.c. of saline containing 0·5 grm. of kaolin. The 'phage-kaolin mixture was shaken thoroughly for ten minutes and left overnight in the ice box. The following morning it was again shaken and centrifugalized at 2,000–2,500 revolutions per minute until the supernatant fluid was clear ; this was free of 'phage. The sediment was re-suspended in 4 c.c. N/100 ammonium hydroxide and again left in the ice box overnight. The next day the suspension was centrifugalized and the supernatant fluid decanted and tested for 'phage and protein. As a rule the fluid was free of even traces of protein, while the 'phage content was at least 50 per cent. of that of the original broth. The above methods were repeated with suspensions of fowl-pox virus using N/500 or N/1,000 ammonium hydroxide as eleutant.

Pirie (98) has also performed adsorption experiments with the Rous sarcoma virus. She found that kaolin, between 0·5 and

2 per cent., would adsorb the virus. Adsorption took place most readily at a neutral reaction as the virus was slightly inactivated at pH 5·0. The virus was eleuted with alkaline phosphate.

Methods employed by Willstätter and his co-workers in the isolation and purification of enzymes have been applied to the study of the virus of poliomyelitis. It has been shown that alumina gel C. mixed with poliomyelitis virus in certain proportions at an acid pH results in the adsorption and inactivation of the virus. Sabin (107) has shown that this adsorption as well as the inactivation is reversible, *i.e.*, by changing the pH to the alkaline side with M/15 Na_2HPO_4 it is possible to free the virus in a state in which it is again capable of producing typical poliomyelitis.

These experiments show that the virus undergoes considerable purification by diminution in the concentration of non-virus-containing substances. Washing the alumina gel C-virus complex with M/5 sodium chloride-M/15 KH_2PO_4 apparently dissociates no virus but is capable of freeing a certain amount of extractible organic substances. Furthermore, it is possible to increase the degree of purity and concentration of the virus by distillation *in vacuo*, and subsequent repeated adsorptions and eleutions. Sabin considers that these methods offer an opportunity for the preparation of a quantity of sufficiently purified and concentrated poliomyelitis virus to warrant the beginning of a study of its chemical nature. They also offer a means of anchoring the virus to an insoluble and centrifugal substance.

Centrifugalization and Temperature Coagulation. It does not seem to be possible to centrifuge suspensions of plant viruses, and even spinning at high speeds does not result in any appreciable concentration of the virus. There is evidence, however, that certain animal viruses are of sufficient density to be shifted by spinning in a tube from the upper to the lower layers of the fluid.

According to Vinson and Petrie (127) the juice of Turkish tobacco plants with mosaic disease contains two well-defined heat-precipitable fractions. One comes down at about 85° C. and the other above 90° C. Only the first fraction contains an appreciable amount of nitrogen. The juice is said to remain infectious after removal of the first fraction, although the virus concentration is greatly reduced.

McKinney (84) states that he was able to remove with comparative ease the extraneous solids and soluble materials from extracts of green tobacco mosaic by centrifuging and temperature coagulation.

Purification by Means of Collodion Membranes. Barnard and Elford (10), using the latter's collodion membranes, have succeeded in concentrating the virus of infectious ectromelia of mice and in washing the suspension free of a proportion of the accompanying proteins. The procedure briefly is as follows: two membranes are used, the pores of one of which are just sufficiently large to allow passage of the virus while the pores of the other hold it back. It has been found possible to concentrate the virus on the second membrane after eliminating all bacteria upon the first. Further washing of the virus residue on the second membrane removes much of the protein material present. This method can also be applied to the purification and concentration of plant viruses, but with more difficulty owing to the presence in plant juices of gums and resins, which tend to plug the membranes to a greater degree than is the case with the animal virus fluids.

As a conclusion to this short account of the purification of virus suspensions, it will be of interest to quote a recent statement of Rivers (104): "As yet it is unlikely that a virus has been obtained in a state of absolute purity. Nevertheless the results already secured are encouraging and should excite further investigations. It may be possible in this way to attain eventually a more accurate concept of the nature of some viruses. For instance, it may be shown that in certain purified virus preparations the number of nitrogen atoms for each infectious unit or particle is insufficient to warrant the supposition that the agents are living, organized structures. Krueger and Tamada (74) have already suggested this viewpoint."

Filtration and Adsorption

For investigations upon the filterability of viruses through porcelain or kieselguhr filters it is necessary to take into con-

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sideration certain factors before any conclusion as to filterability can be drawn. The more important of these factors are the *p*H of the virus-containing fluid, the state of purification of the virus suspension, the pressure exerted and possibly the electrical charge of the virus or of the particles to which the virus may be adsorbed. The question of adsorption plays a very large part in virus filtration, and especially so in the case of candle filtration where there is available so large a bulk of material liable to adsorb the virus. On this point Mudd (86) states: " The contact surfaces of the pores of a Berkfeld filter and the fluid bathing them are the site of an electrical potential difference, an ordinary Helmholtz double layer, the solid walls carrying a negative and the liquid a positive charge. We should predict, therefore, that when solutions or suspensions are filtered, positively charged particles would be adsorbed, at least to some extent, on the filter walls and thus removed from solution, while negatively charged particles would be borne through on the filtration stream. This expectation we find fulfilled with dyes. Solutions of ' basic ' dyes like methylene blue in which the coloured ion is electro-positive may be completely decolorized by the filter at first, only the latter portions of the filtrate being coloured by the dye. Solutions of ' acid ' dyes like eosin in which the coloured ion is negative, pass through a Berkfeld candle without demonstrable change."

In reviewing the filterability of plant viruses by candle methods it will be seen how widely the various viruses differ in this respect. This variation is more probably due to reasons connected with adsorption and electrical charge than to mere size of the virus particle. The following examples will demonstrate this: the writer has shown that a certain potato virus, Y, will not pass an L_3 Pasteur-Chamberland candle which has an approximate pore size of 2·5 μ; the same virus, however, readily passes a collodion membrane of average pore diameter 0·425 μ (116), and the same is true of the virus causing spotted wilt of tomatoes. Similarly, the causative agent of bovine pleuro-pneumonia easily passes a Pasteur-Chamberland L_2 candle, but this grade of candle constantly prevents the passage of vaccine virus (121, p. 19), which is reputed to have a particle size of 0·15 μ (36). According to

Elford (*in litt.*), however, vaccinia virus, in suitable medium and at the right pH, filters readily through such a candle.

The virus-containing sap from affected plants is best cleared of visible particles and prepared for candle filtration by preliminary passage of a sand-and-pulp filter. This consists of a short glass cylinder about 5 in. in length and 1 in. in diameter, narrowing at the neck into an ordinary delivery tube which passes through a rubber bung in a suction flask. At the bottom of the cylinder is placed a pledget of non-absorbent cotton wool, then comes a tightly packed layer of macerated ashless filter paper; this is followed by a layer of about 2 in. of acid-washed sand and finished off with a further layer of paper pulp. Passage of the sap through this filter gives a clear brown fluid free of visible particles.

For extracting the sap from infected plants the writer uses a screw press, and the juice thus obtained is used undiluted. In filtration experiments with tomato mosaic viruses Henderson Smith (113) adds about 300 c.c. of distilled water per 100 grm. of tissue; this yields between 330–340 c.c. of the crude fluid after squeezing through muslin. Juice so treated may be regarded as diluted 1 : 8–1 : 10 before passing through the candles.

The following data are given regarding the filterability of certain representative plant viruses:—Iwanowsky, working with tobacco mosaic, was the first to show that this virus would pass through a filter candle. Since his time the virus of tobacco mosaic has been filtered on very many occasions and has been shown to be capable of passing various grades of the Pasteur-Chamberland and Berkfeld candles.

Henderson Smith (113) found that the virus of aucuba mosaic of the tomato was easily filterable through an L_3 Pasteur-Chamberland candle. K. M. Smith, working with another tomato mosaic virus (115), has obtained positive infections with this virus after passage of all grades of Pasteur-Chamberland candles from L_1 to L_{13}. The writer has also found that the potato virus X will pass an L_3 and an L_5 candle, but the potato virus Y and the virus of tomato spotted wilt will not pass an L_3 candle.

Folsom (43) reports inactivation of potato rugose mosaic by passage of the coarsest grade of Berkfeld filter. Rugose mosaic of potato is caused by a virus complex (71) and the inactivation referred to by Folsom is due to the elimination on the filter of the virus Y, which is a constituent part of rugose mosaic and which will not pass a coarse Berkfeld filter.

Henderson and Wingard (54) state that the virus of tobacco ringspot is filterable through a Berkfeld filter of W grade (W = pore size, 3–4 μ), provided that the juice is first freed of its suspended solid matter, while Priode (101), dealing with apparently the same ringspot virus, finds that it will not pass a Berkfeld filter, grade N (N = pore size, 5–7 μ) (Mudd *in* 103). The writer has carried out some experiments on membrane filtration of this same ringspot virus, which was kindly supplied by Dr. Price. It has been found that the virus just passes a collodion membrane of average pore diameter of 0·35 μ but is held back by a membrane of average pore diameter of 0·194 μ.

There are two cases of viruses which are not sap-transmissible in which the filterability has been investigated by means of the special technique of feeding insects on virus suspensions which is described in Chapter V. Storey (118) has demonstrated that the virus of streak of maize, which is transmissible only by the leaf-hopper, *Cicadulina mbila,* will pass, at a *p*H of about 6, an L_1 Pasteur-Chamberland candle, Berkfeld V and N filters, less freely through an L_3 candle and not at all through a Seitz E.K. filter disc. By similar means Severin and Swezy (110) have shown that the virus of sugar-beet curly top is filterable through coarse, medium and fine Berkfeld candles.

The viruses causing the following diseases are apparently not filterable by candle methods: the filters which have been used in each case are indicated in brackets—mosaic of dock (Jenkins filter, English Berkfeld candle, Grainger and Cockerham (51)); mosaic of bean (coarse Berkfeld candle, Fajardo (39)); calico disease of potato (Porter (100)); bunchy-top of tomato (L_1 Pasteur-Chamberland candle, Seitz E.K. 6, McClean (82)); spotted wilt of tomato (Pasteur-Chamberland candles, L_1, L_3, L_5, Smith, K. M. (115)); the Y potato virus (Pasteur-Chamberland candles L_3 and L_5, Smith, K. M. (116)).

It is unlikely that the failure of these viruses to pass the various filter candles is due to the size of the respective particles, and it is probable that such failure is due, at all events in some cases, to adsorption of the virus on the candle. Furthermore, two of the above-mentioned viruses, *i.e.*, potato virus Y and that of tomato spotted wilt, have been shown by the writer to pass through a collodion membrane with an average pore size very much smaller than the average pore size of the candle which held back these two viruses. Again, in experimenting with a mixture of the two potato viruses X and Y, in plant sap, the writer has shown (116) that passage of such sap through a kieselguhr bed removes the Y virus by adsorption while the X virus is recovered in " pure culture " in the filtrate.

The following data regarding the filterability of representative animal viruses are given for comparison with the foregoing: the grade of filter through which the virus will pass is given in each case: feline distemper, Berkfeld N (59); yellow fever, Chamberland F, Berkfeld V and N, but not apparently through Berkfeld W candles (117); vaccinia, Chamberland F and Berkfeld V, N and W (24); foot-and-mouth disease, Berkfeld V and N candles and through Chamberland candles down to L_5 (92); the virus of fowl-pox passes a Berkfeld V candle with a reduction in potency, but is retained by Chamberland L_2 candles (78). It should perhaps be mentioned for the sake of comparison that the Chamberland F filters are equivalent to the Chamberland L_5. As regards the Berkfeld filters, the pore size of the W grade is about 3–4 μ, of the N grade about 5–7 μ and of the V grade about 8–12 μ (Mudd *in* 103, p. 57).

Filtration by Means of Collodion Membranes. Collodion filters have been largely used in virus studies in order to avoid the numerous disadvantages possessed by porcelain or kieselguhr candles. Bechhold prepared collodion filters by soaking filter papers in a solution of gun-cotton in glacial acetic acid and removing the acid subsequently by washing in distilled water.

Collodion filters have been employed previously in plant virus studies by Duggar and Karrer (33), by Mulvania (87) and by Klebahn (67), and in animal virus studies, among others, by Olitsky and Boëz (92).

A somewhat detailed account will be given here of the preparation of graded collodion membranes according to the technique developed by Elford, and some of their applications to the study of plant viruses will be indicated. Extracts from the original papers are freely quoted.

Elford's investigation of the physical properties of collodion films revealed the fact that they can exhibit two very definite types of structure : "(*a*) *microgel*, a relatively coarse structure of microscopic order, and (*b*) *ultragel*, the structural elements of which are only resolvable by ultra-violet light photography which shows them to be built up of particulate matter. These facts explain the difference in behaviour between acetic acid-collodion membranes on the one hand, and ether-collodion membranes on the other. In the former type of membrane the microgel and ultragel structures co-exist, thus giving a pore size which ranges between rather wide limits. Ether-collodion membranes, however, possess only the ultragel structure, and although uniform are not sufficiently permeable for general filtration work. In a previous optical study of ether-alcohol collodion films, two facts were established : (1) the ultragel structure is consequent upon the spontaneous gelation of the collodion prior to the washing process in water, the latter serving to fix the already potentially existent structure ; (2) the ultragel is built up of particulate material." Elford's line of attack was an endeavour to increase progressively the state of aggregation of the nitrocellulose in the collodion while still retaining the spontaneous gelling properties of the system. "It is known that the addition of certain non-solvents does produce an increase in membrane permeability. Successive additions of small amounts of water up to 5 per cent. produce a gradual uniform increase in permeability, but beyond this concentration the nitro-cellulose becomes completely coagulated. Amyl alcohol and acetone were found to be mutually antagonistic in their solvent action towards nitrocellulose, for, while each in conjunction with ethyl or methyl alcohol is an excellent solvent, the presence of both in the same solvent medium eventually results in the coagulation of the nitro-cellulose." Elford has exploited this phenomenon to bring about the desired aggregation in the collodion used for preparing ultra-filter membranes. Varying amounts of amyl alcohol were added to a 10 per cent. solution of Scherings' celloidin in ether : alcohol : acetone (1 : 1 : 2 parts by weight) and films were prepared and studied. It was found, as expected, that the collodion films increase in permeability as increasing amounts of amyl alcohol are incorporated. These new membranes have been named by Elford, *Gradocol* membranes, since they are products of a graded coagulation of collodion.

The writer proposes to describe, step by step, the making of a gradocol membrane, while the reader is referred to the original paper by Elford (34) for exact details regarding the technique and for reasons for the precise procedure followed.

The whole process of preparing the membranes must be carried out in a constant temperature chamber at a temperature of 22·5° C., and all glassware and vessels used in the process must be of this same temperature.

A standard ether-alcohol collodion is used, prepared by Nobel Chemical Finishes Ltd., of Slough, and known as Necol (No. 356.A/9). The necol is mixed with its own weight of acetone, which should have been dried over potassium carbonate and distilled. This is the necol stock solution, and the necol is best kept in this way. " A " c.c. of amyl alcohol (B.D.H. " A.R.") are added per 40 grm. necol stock solution, and this system is then diluted with its own weight of a mixture of ethyl alcohol (dried over quicklime and distilled) and ether (dried over metallic sodium and distilled), which are in the ratio 1 : 9 parts by weight. The " a " c.c. of amyl alcohol can be varied from 4 to 10 or upwards accordingly, as it is desired to increase the permeability of the membrane. Such a system is described by the nomenclature N.a/40 (1 : 9).

The next step is to shake the collodion system for a standard period in a mechanical shaker ; Elford gives the time necessary as four hours, but the writer has found that two hours' shaking also gives good results. After shaking, the collodion is poured into a special cell previously prepared for evaporation, and here it will be necessary briefly to describe this evaporation cell. Two squares of plate glass, ground flat and polished, are cemented together by means of egg-white, one square of glass having a circular hole either 20 or 40 cm. in diameter. The apposition of these two squares thus gives a shallow cell about 1 cm. deep and 20 or 40 cm. in diameter, according to the size of the hole cut in one of the sheets of glass. The cell is then arranged on a stand with three adjusting screws and carefully made level by means of a spirit level and the adjusting screws.

The exact volume of collodion required is poured into the cell, which is surrounded with a paper collar to exclude draughts, and the evaporation period carefully timed. When the requisite time for evaporation has elapsed the membrane is flooded in the cell with distilled water. Coagulation then sets in and the film becomes white and opaque in appearance. Washing with continued changes of distilled water must be continued for fourteen days in order to remove the last traces of amyl alcohol.

After the membrane is set, it may be left in water in a large crystallizing dish for twenty-four hours and should then be cut into the requisite discs to fit the Elford ultra-filter (see Fig. 9) with a steel punch of the required size. In cutting the discs from the large membrane a margin of *at least* 2 cm. round the edge should be discarded.

As regards sterilization, the membranes will not stand autoclaving, but they may be steamed. The steaming should be carried out before the membranes are standardized. Afterwards they may be kept in sterile water in small sterile crystallizing dishes. Elford suggests the alternative method of keeping the films sterile by carrying out all the washing processes in the sterile atmosphere of an ultra-violet light chamber.

Standard conditions for the preparation of fairly permeable membranes are as follows, and these conditions must be strictly adhered to :—

Volume of collodion, N.a/40 (1 : 9) .	75 c.c.
Diameter of cell	20 cm.
Temperature	22·5° C.
Evaporation period	75 min.

Elford finds that these conditions give membranes with an average pore diameter varying from 0·60 μ to 0·81 μ accordingly as "a" c.c. of amyl alcohol represents 4, 6, 8 or 10 c.c. It must be pointed out, however, that these values seem to depend somewhat on the original necol used in the preparation of the parent collodion. Elford has shown that the permeability can be varied within narrow limits by varying the evaporation period of the collodion, the permeability decreasing as the evaporation period is prolonged. For producing wider variations in permeability, additions of water or acetic acid in cubic centimetres per 100 grm. of the collodion system must be made. The water very greatly increases the permeability of the resulting membrane, while the acetic acid decreases it. It is important that the solution be shaken for the standard period after each percentage addition, in order to ensure homogeneity.

Having prepared the collodion membrane, it must then be standardized. Elford obtains his data from certain tests which form a definite part of routine membrane standardization. These tests consist of (1) rate of flow of water; (2) average pore diameter; (3) critical air pressure. Only one of these tests, the average pore diameter, will be dealt with here. This figure is calculated from the rate of flow of water data and a determination of the water content of each particular grade of membrane. The formula used is based upon the assumption that Poiseuille's law governs the rate of flow of water through the pores of the membranes.

$$r = 2l\sqrt{\frac{2q\eta}{pv}},$$

where r = radius of pores in cm.
l = length of capillary thickness of membrane in cm.
q = volume of water passing in c.c./sec.
η = viscosity of water at temperature of measurement in C.G.S. units.
p = pressure producing flow in dynes/sq. cm.
v = total volume of pores (assumed equivalent to water content of membrane).

The apparatus devised by Elford for measuring the rate of flow of water is shown in Fig. 9. It consists of two narrow glass necks with the shoulders ground smooth—two glass bottle necks suitably ground with carborundum may be used—into which are fitted, by rubber bungs, the calibrated glass tubes shown in the figure. The glass necks are held firmly together by means of an ebonite clamp.

It is important to observe certain precautions in using this apparatus. All the glassware must be perfectly clean and free from any trace of grease; it should be kept in sulphuric acid-bichromate solution when not in use. The rubber bungs should be coated with a thin layer of paraffin wax to prevent particles of rubber interfering with the rate of flow of water. The cell should be submerged in water at a constant temperature of 20° C. during the experiment.

In a more recent paper Elford (35) discusses the principles of ultrafiltration as applied in biological studies: "An ultrafilter membrane does not behave like an ordinary sieve in which coarseness of mesh alone determines whether or not a non-deformable particle shall pass. As the meshes become smaller, the relative importance of the thickness of the sieve increases, until eventually, as in the case of a membrane, the length of a pore becomes very much greater than its diameter. The liquid traversing the pores of a membrane is in contact with a very large surface, and hence surface phenomena may be expected to play an important part in an ultrafiltration process. The ratio, area of pore surface/pore volume, varies inversely with the first power of the pore radius, and hence becomes very large for ultrafine capillaries."

"Lateral diffusion between the mainly effective capillaries occurs very slowly. The ultrafilter membrane may thus be regarded from the point of view of its performance as a porous structure, the effective pores being long and relatively straight channels, formed by the communicating interstices between the elements in superimposed strata of the aggregated nitrocellulose particles."

"There are two stages in the filtration of a colloidal solution. Stage 1 involves the adjustment of equilibrium between the surface of the capillaries and the solution, while stage 2 consists of true

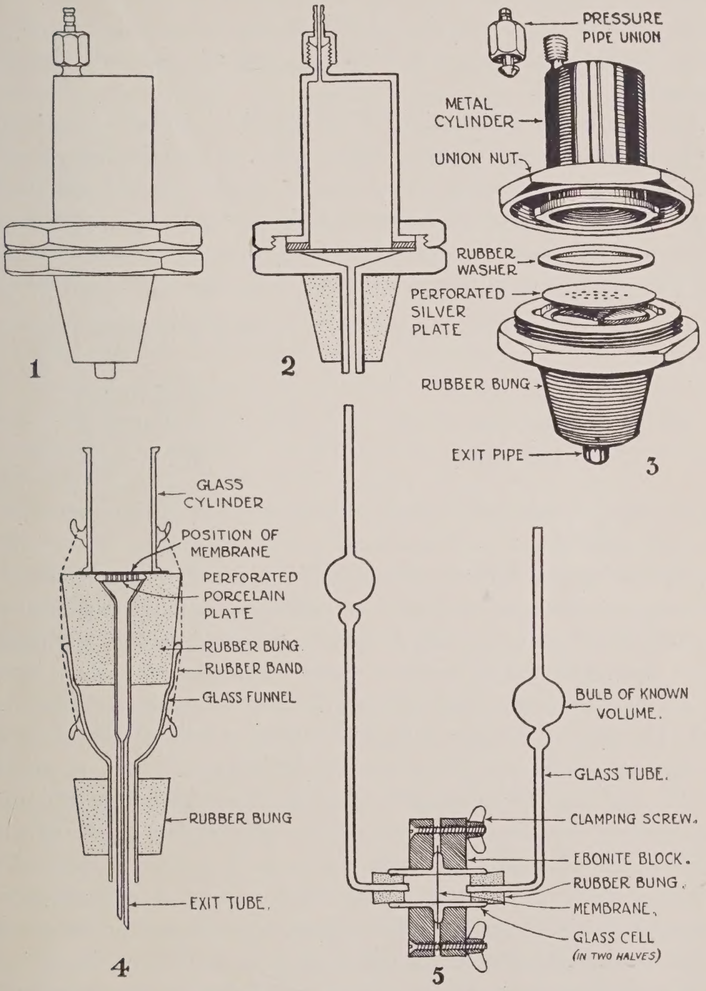
PRESSURE PIPE UNION
METAL CYLINDER
UNION NUT
RUBBER WASHER
PERFORATED SILVER PLATE
RUBBER BUNG
EXIT PIPE
1
2
3
GLASS CYLINDER
POSITION OF MEMBRANE
PERFORATED PORCELAIN PLATE
RUBBER BUNG.
RUBBER BAND
GLASS FUNNEL
RUBBER BUNG
EXIT TUBE.
4
BULB OF KNOWN VOLUME.
GLASS TUBE.
CLAMPING SCREW.
EBONITE BLOCK.
RUBBER BUNG.
MEMBRANE.
GLASS CELL (IN TWO HALVES)
5

Fig. 9.—Nos. 1, 2, 3. Elford's ultrafilter for use with collodion membranes under positive pressure. No. 4. "Der Buwa-Apparat," a filter of German design for use with collodion membranes under negative pressure. No. 5. Elford's apparatus for measuring the rate of flow of water in standardizing the Gradocol membranes (see p. 78).

filtration through the modified, equilibrated membrane. The filter surface has a certain adsorbing capacity, and this affinity must be satisfied before unhindered passage through the filter occurs."

As regards the estimation of the particle size of viruses from their filtration end-points, Elford has adopted the practical procedure of expressing the probable limits for the size of the smallest particle just retained by a given membrane, as obtained *under the best available conditions for filtration.* The relationship between particle and pore size is given in the following table :—

Membrane, average pore diameter.	*Size of retained particle.*
μμ	
10–100	(0·33–0·5)d
100–500	(0·5–0·75)d
500–1,000	(0·75–1·0)d

where " d " equals the average pore diameter of limiting membrane for optimum filtration conditions.

Application of Elford's Technique to Plant Virus Studies. A considerable amount of work has been carried out by Elford and his colleagues on the study of certain animal viruses by means of these membranes, and some of their results will be given briefly in a later paragraph dealing with the size of virus particles.

At the time of writing Elford's technique has only been applied to the study of plant viruses by MacClement and Henderson Smith and by the present writer, and some of the difficulties experienced by these workers in dealing with viruses in plant extracts are discussed herewith.

Elford finds that if the determined values of rate of flow of water are plotted against the average pore diameter values it is possible to read off directly the average pore size values of membranes from determinations of rate of flow of water. MacClement and Henderson Smith and the writer have found it advisable up to the present to standardize every membrane individually before use. It has also been found (81) that very small local or general differences in atmospheric humidity, slight currents of air, etc., affect the final product to a surprising extent. There may be marked difference of pore size between the central area of a membrane and the portions lying near the rim, and it is therefore essential to discard at least 2 cm. of the periphery. These difficulties emphasize the importance of a most painstaking attention to detail.

Another difficulty is the rapid clogging of the pores of the membrane during filtration by the tannins, resins and other readily precipitated materials which do not occur in animal tissues. Such clogging, which varies in intensity with different plant species, occurs even after the most careful clarification of the plant juice containing the virus, and thus affects the results as a guide to the size of the virus particle. To this clogging is also presumably due the gradual diminution of the quantity of virus passing through the membranes instead of passing in undiminished quantity through the series of membranes down to a definite end-point at which no more virus passes.

The writer has applied this collodion technique to the study of a number of plant viruses, and more particularly to the two potato viruses known as X and Y. These two latter differ from one another very materially in symptom expression, physical properties and relationship with insect vectors. It has been shown (116) that while the X virus passes both an L_3 and an L_5 Pasteur-Chamberland candle, the Y virus will not pass an L_3 candle. On the other hand, experiments have proved that *both* viruses will pass a collodion filter prepared according to Elford's technique of average pore size of 0·425 μ, while both are held back by a membrane of average pore size of 0·35 μ, compared with a pore size in the L_3 candle of about 2·5 μ. The virus suspensions used in these experiments consisted of undiluted sap from infected tobacco plants at *p*H values of 5·7–5·9. It is probable that this inability of the Y virus to pass the candle is not directly concerned with the size of the virus particle, but is due to adsorption of the virus by the candle.

The main difficulty in the way of a successful application of Elford's collodion filtration technique to plant virus studies lies in the liability of the membrane to be clogged by the proteins, mucilage and tarry materials which are to be found in the expressed sap of many plants, particularly tomato and tobacco. If sap, extracted from a tobacco plant and cleared of all visible particles by passage of a sand-and-pulp filter and a coarse membrane, be allowed to stand overnight at room temperature, it will show a heavy precipitate in the morning. It is evident that some method of separating the virus from the proteins, resins, etc., in the sap is needed to allow of a wider application of Elford's technique in plant-virus studies.

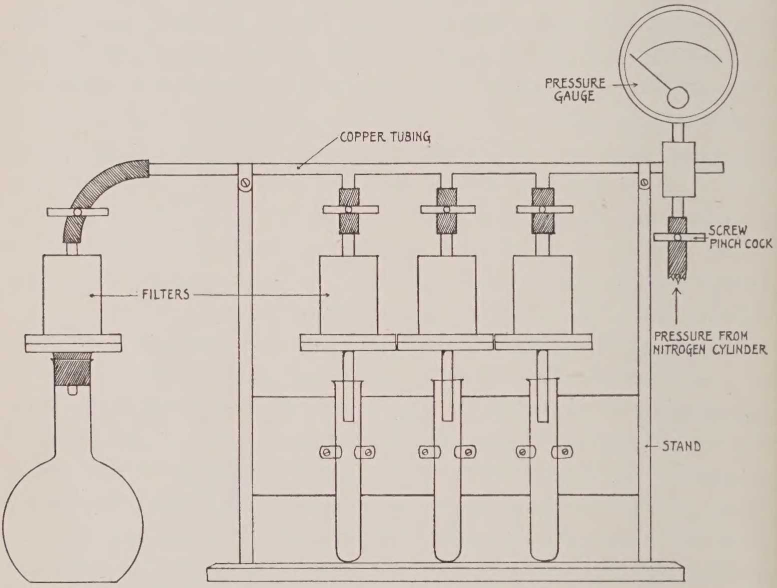

Fig. 10.—A simple arrangement devised by W. D. MacClement for filtration, in one operation, of a virus through a series of collodion membranes of different average pore diameter.

Size of Virus Particles

There are various methods by which the size of minute particles may be calculated. First, there is the method of direct measurement by means of the microscope provided the object is capable of resolution ; the diameter of the smallest particle visible under a good oil-immersion lens lies between 0·1 μ and 0·2 μ (1,000 $\mu\mu$ = 1 μ = 0·001 mm.).

The true size and form of any object whose dimensions are less than half of the wavelength of the light used to illuminate it cannot be correctly observed on account of the diffraction which takes place. The wavelengths of the rays of the visible spectrum lie between 400–700 μ; the invisible ultra-violet rays to which the photographic plate is sensitive are much shorter (26A). Barnard, with his ultra-microscope and using these invisible rays, has measured the particle size of several animal viruses and bacteriophages, infectious ectromelia of mice for example. This work will be referred to in a subsequent paragraph.

Other methods of measuring the size of virus particles are by means of filtration and ultrafiltration where the factors influencing the passage of particles through pores of graded diameters are known and controlled, and by diffusion and centrifugation.

Rivers has recently reviewed the subject of the size of virus particles, and the following data are taken from his review (104).

Hæmoglobin. For a number of years the molecule of hæmoglobin was thought to be 30 $\mu\mu$ in diameter. Recently, however, figures derived from the results of Svedberg and Nichol's centrifugation experiments (120) and Northrop and Anson's diffusion experiments (90) with hæmoglobin indicate that its diameter is approximately 5·5 $\mu\mu$. Many estimations regarding the magnitude of viruses have been based on the former figure for the diameter of the hæmoglobin molecule, 30 $\mu\mu$. If this figure is incorrect, many statements concerning the size of viruses are also inaccurate.

Mosaic Virus. Duggar and Karrer (33), by means of ultrafiltration, found the infectious particles of (tobacco) mosaic virus to be of the same order of magnitude as hæmoglobin molecules, namely, 30 $\mu\mu$ in diameter. Vinson (128), however, says that Duggar's experiments interpreted in the light of recent work regarding the size of hæmoglobin molecules indicates that the diameter of mosaic virus is about 5·5 $\mu\mu$.

Herpetic Virus. Zinsser and Tang (130), by means of ultrafiltration, estimated the diameter of herpetic virus to be 20–100 $\mu\mu$.

Levaditi and Nicolau (79) in the same way found that the virus passed through membranes which retained toxins, hæmolysins, complement and serum globulins. Bedson (13), however, unable to confirm Levaditi and Nicolau's results, obtained evidence by centrifugation that herpetic virus is probably of sufficient size to be visible.

Foot-and-mouth Disease Virus. Olitsky and Boëz (92), using ultrafiltration, found that the virus of foot-and-mouth disease is 20–100 $\mu\mu$ in diameter. Elford, by means of his special membranes, estimated it to be 8–12 $\mu\mu$.

Poliomyelitic Virus. By means of ultrafiltration Krueger and Schultz (72) in 1929 found that the virus of poliomyelitis possesses a magnitude not greater than 300 $\mu\mu$. In 1931, by the same means, Clifton, Schultz and Gebhardt (25) obtained results indicating that the diameter of the virus lies below 50 $\mu\mu$.

Fowl-plague Virus. By means of ultrafiltration Andriewsky (4) secured a figure of 2·5 $\mu\mu$ for the diameter of fowl-plague virus, while Bechhold and Schlesinger (11) by centrifugation found it to be 120–130 $\mu\mu$.

Bacteriophage. According to d'Herelle (56), who used ultrafiltration, the diameter of the bacteriophage is approximately 30 $\mu\mu$. Krueger and Tamada (73), by means of purified bacteriophage preparations and ultrafiltration, found it to be 5 $\mu\mu$; and Hetler and Bronfenbrenner (57), by means of a diffusion method, estimated it to be 1·2–22·8 $\mu\mu$.

Rous Virus. According to Zinsser and Tang (130), the Rous virus is 20–100 $\mu\mu$; according to Mendelsohn, Clifton and Lewis (85), 50 $\mu\mu$; according to Fränkel (44), 10 $\mu\mu$. All these workers obtained their figures by means of ultrafiltration.

Vaccine Virus. Levaditi and Nicolau (79) reported that vaccine virus passes through membranes impervious to toxins, hæmolysins, complement and serum globulins. Bland (17), however, from the results of his centrifugation experiments, not only concluded that Levaditi and Nicolau's findings were incorrect, but that vaccine virus is probably large enough to be seen. Bechhold and Schlesinger (11), by means of centrifugation, estimated that the active agent is 210–230 $\mu\mu$ in diameter, while Yaoi and

Kasai (129), working with "purified" virus, found that it diffused at the rate of fuchsin particles and is therefore not capable of being seen.

Rivers comments on these data as follows: "One cannot consider the results cited above without being amused and dismayed. Many of the workers seemed in no way concerned about the possibility that they might have been estimating, not the magnitude of viruses, but the size of particles of degraded cells to which the viruses were attached." He sums up by saying: "Unfortunately, none of the figures can be accepted without reservations. At present the exact size of no virus is known."

It remains to consider some more recent results of studies upon the size of certain plant and animal viruses as measured by means of Elford's membranes and Barnard's ultramicroscope and to compare their measurements with the foregoing data.

As regards plant viruses, MacClement and Henderson Smith (81), using Elford's technique, obtained the following results. The virus of a tobacco mosaic (Johnson No. 1) passes the 0·051 μ membrane, though in reduced quantity (only four plants infected out of eight), and passes the 0·154 μ grade easily. The virus of yellow tobacco mosaic (Johnson No. 6) is of the same size, passing 0·051 μ (two plants positive out of eight). Aucuba mosaic virus passes 0·120 μ and 0·112 μ, but does not pass 0·10 μ, 0·06 μ or 0·051 μ. A virus from *Hyoscyamus* passes 0·30 μ, but not 0·234 μ or any smaller membrane. By Elford's method of calculation (see p. 80) these figures would indicate a particle size of 15 $\mu\mu$ for the tobacco and yellow mosaic viruses, about 40–50 $\mu\mu$ for aucuba mosaic virus and 150 $\mu\mu$ for the *Hyoscyamus* virus. The value found for tobacco mosaic virus comes midway between Duggar's estimate of 30–40 $\mu\mu$ and the recent estimate of 5 $\mu\mu$ by Waugh and Vinson (128A).

Using Elford's membranes, the writer has measured the approximate particle size of four plant viruses. These are the potato viruses X and Y, the virus of tomato spotted wilt and a ringspot virus from America, kindly supplied by Dr. Price.

It has been found that the X virus passes a 0·425 μ membrane, but not one of 0·33 μ. The Y virus passes a 0·425 μ membrane also and is stopped by 0·35 μ. The tobacco ringspot virus from America just passes a 0·35 μ membrane, but is stopped by a 0·194 μ. The virus of tomato spotted wilt passes a membrane of average pore size 0·35 μ, but does not pass a 0·22 μ membrane. In this last case an extra factor has to be considered—namely, that during the process of filtration the virus is all the time losing potency because of its very short life *in vitro* (see p. 59). By Elford's method of calculation the approximate particle sizes of the X and Y viruses are as follows :—

X has an average pore diameter of 0·20 μ and Y an average pore diameter of 0·25 μ.

As regards recent work on the measurement of particle size of animal viruses, Barnard and Elford (10), working with the virus of infectious ectromelia of mice, have measured the particle size by two methods—Barnard by means of his ultramicroscope and ultra-violet light, and Elford by his membranes.

Using a wavelength of 0·275 μ, Barnard obtained by this means good photographic images of the particles in the inclusion bodies, and measurements of them indicate a size ranging from 0·13 μ to 0·14 μ. Elford, using his membranes, arrives at a closely similar figure of 0·10–0·15 μ.

Working with vaccinia virus, Elford and Andrewes (36) state that filtration experiments through Gradocol membranes suggest that vaccinia virus particles have a diameter of 0·125–0·175 μ (cf. Bechhold and Schlesinger's computation by centrifugalization of 0·210–0·230 μ).

Galloway and Elford (47) find that the diameter of the virus particles of foot-and-mouth disease lies between 8–12 μμ.

These findings differ considerably from those of Olitsky and Boëz (92), who estimated the size of foot-and-mouth disease virus to be between 20–100 μμ.

Elford and his colleagues (38) have recently filtered the virus of herpes through graded collodion membranes, and they find the end-point of filtration to be 0·2 μ in membrane grade. This leads them to assign a probable size to the herpes virus particles of 0·1–0·15 μ. This is the same size as that given for the elementary bodies of infectious ectromelia of mice, and, since these bodies have been photographed by Barnard (10) using ultra-violet light, it follows that the herpes virus may also come within the scope of ultra-violet light photography. The size values of 0·1–0·15 μ given by Elford *et al.* for herpes virus agree fairly closely with the values of 0·10–0·02 μ given by Zinsser and Tang (130) for the same virus.

Finally, Elford and Andrewes (37) have examined the size of different bacteriophages and a statement of theirs may be quoted here as relevant : " There are many references in the literature to work on the size of ' the bacteriophage ' ; it would be as reasonable to talk of the size of ' a bacterium,' for there can be very few workers to-day who would agree with d'Herelle that all races of bacteriophage are representatives of a single species, the *Bacteriophagum intestinale*."

Elford and Andrewes find that the various 'phages appear to differ from one another in size, but the particles of any one 'phage seem to be very uniform in size and do not vary even when the 'phage is grown on different organisms. The estimated diameter of the smallest 'phage studied was 8–12 μμ and that of the largest was probably between 50–75 μμ. These conclusions as to the relative sizes of the different 'phages were confirmed by diffusion experiments. It will be recalled that the measurements of bacteriophage particles quoted by Rivers were as follows : d'Herelle, 30 μμ ; Krueger and Tamada, 5 μμ ; and Hetler and Bronfenbrenner, 1·2–22·8 μμ.

In conclusion to this section on the size of virus particles the comparative table on p. 88 is reproduced (Elford (35)).

Particulate Nature

The concept of a virus as a particulate body depends largely on the theory held as to the ultimate nature of viruses. One can hardly doubt, however, that the viruses are corpuscular bodies in view of the foregoing data on the sizes of virus particles. The only other alternatives are the suggestion of Beijerinck of a living contagious fluid and that of Sanfelice of a non-living poison, elaborated by the affected cells.

The work of Elford and others on the measurement of virus particle size by ultrafiltration and the photographs taken by

Substance.	End-point.	Probable Size by Filtration.	Size by other Methods.
	μ	μ	
B. prodigiosus	0·75	0·5–0·75	0·5–1·0 μ microscopically.
Bovine P. pneumonia—			
Spheres	0·35	0·175–0·25	0·2–0·5 μ microscopically (Barnard).
Particles	0·20	0·1–0·15	>0·2 μ
Vaccinia virus	0·25	0·125–0·175	0·21–0·23 μ centrifugal analysis (Bechhold and Schlesinger, 11). 0·17–0·18 μ (max.) ultra-violet light photography (Barnard).
Infectious ectromelia virus.	0·2	0·1–0·15	0·13–0·14 μ ultra-violet light photography (Barnard, 10).
Herpes virus	0·2	0·1–0·15	
	μμ	μμ	
Gold sol (purple)	80	30–40	50–60 μμ ultramicroscopical count.
'Phage C 36	65	20–30	Bechhold and Villa (12). *B. coli* 'phage, 35 μμ optical method. Hetler and Bronfenbrenner (57), *B. coli* 'phage 22·8–1·2 μμ by diffusion.
Gold sol (red)	40	15–20	20 μμ ultramicroscopical count.
Foot-and-mouth disease virus.	25	8–12	
Oxyhæmoglobin	10	3–5	5 μμ ultracentrifugal analysis (Svedberg, 120). 5·4 μμ diffusion (Northrop and Anson, 90).
Egg albumin	6	—	4·34 μμ ultracentrifugal analysis (Svedberg, 120).

Barnard by means of ultra-violet light of the apparent virus organisms would appear to be proof enough of the particulate nature of viruses, although the possibility must be borne in mind that the bodies in question are not the actual virus particles but carry the virus adsorbed on to them. The same objection of adsorption to protein particles could be raised to the evidence in favour of the particulate nature of viruses which is afforded by centrifugalization of animal virus suspensions, where spinning in a tube will shift the virus from the upper to the lower layers of the fluid.

Further evidence in favour of the biological- and consequently particulate-nature of viruses is that afforded by the recent description of an apparent life-cycle of the psittacosis virus given by Bedson and Bland (see p. 35).

Takahashi and Rawlins (123) have attempted to obtain evidence regarding the shape of virus particles by the use of polarized light, using methods similar to those of Ambronn and Frey (3) for determining the shape of colloid particles. An apparatus to attempt this was designed and tested. The supernatant liquid from centrifuged juice of frozen mosaic tobacco plants (Johnson's tobacco virus 1) was examined by means of this apparatus and compared with similarly treated juice from healthy tobacco plants. The virus sap was seen to be distinctly doubly refractive and appeared as a bright streak across a dark field, while the sap from healthy plants showed no double refraction. It is considered that the occurrence of stream double refraction in the juice of mosaic plants, its absence in the juice of healthy plants and its similarity to the stream double refraction shown by sols containing rod-shaped particles, indicate that the virus of tobacco mosaic, or some substance regularly associated with it, is composed of rod-shaped particles.

In a more recent statement Takahashi and Rawlins (124) find that centrifuged juice from *unfrozen* healthy leaves also exhibits stream double refraction. Similar juice from healthy leaves, after treatment with Vinson's safranin-Lloyd's reagent precipitation, failed to show double refraction, even when diluted with 200 parts of water. It is concluded that the present evidence is insufficient to warrant assumptions as to whether the virus particles are or are not responsible for all or part of the double refraction exhibited by juice from diseased plants.

By making use of the local lesions formed on leaves of *Nicotiana glutinosa* by the virus of tomato aucuba mosaic, Caldwell has counted the spots formed after inoculation with virus juice diluted to different strengths. From the data obtained, Caldwell considers that there is strong presumptive evidence that there is a direct and simple relationship between the dilution and the number of spots when equal amounts are used on each leaf. The inference drawn is that the virus agent is apparently particulate in nature and that each particle, or group of particles, is able to induce the appearance of a necrotic spot on the leaf of *N. glutinosa*, if entry into a cell be effected. It has also been shown that the destruction, by digestion with trypsin, of the proteins in a suspension of tomato aucuba mosaic virus does not increase the number of local lesions formed on inoculation. It would therefore appear from this that the virus is not aggregated into masses which easily break down mechanically, nor is it adsorbed in groups on to the proteins of the plant (22).

Boycott's conclusion on the particulate nature of viruses may be quoted here : " They must be ultimately particulate because all matter is so arranged, and from the readiness with which they are adsorbed on to appropriate surfaces the particles are presumably much larger than the molecules of simple salts " (20).

Electric Charge of Virus Particles

Takahashi and Rawlins (122) have carried out some experiments to determine the electric charge of the virus of tobacco mosaic. The results of these experiments show that the unpurified virus migrates to the anode in an electrical field at *p*H values between 4 and 9, but no migration occurred at *p*H values between 3 and 1·2.

Olitsky and Hoffmann (94) have made a study of tomato mosaic virus with the objects of noting (1) the possible migration of a plant virus in an electric field ; (2) the direction of migration ; (3) any difference in behaviour of filtered and unfiltered suspensions. The results show that the mosaic virus, or particles containing it, migrate to the anode in an electrical field at *p*H readings of 5·3–8·5, the tomato virus therefore agreeing in this respect with most viruses of mammalian origin and with bacteriophage. According to Birkeland (16) the direction of migration of plant viruses in an electric field is not altered by the state of purity of the virus suspension nor by the method of purification. He found that the crude viruses of tobacco mosaic, spot necrosis, ringspot and attenuated mosaic migrated to the anode—that is, they behaved as though negatively charged. The same viruses, purified by the methods of Vinson and Petrie (see p. 66) and of

Brewer, Kraybill and Gardner (21), also migrated to the anode. The purified preparations were biuret-negative.

Bolas (19) has also experimented on the movement of a tomato mosaic virus in an electric field. His tentative conclusions are that the portion of the virus producing severe symptoms is only to be found in the middle and towards the positive pole, while a portion capable of producing only mild symptoms is to be found on the negative side of the mid-point.

For comparison with the above, the electric charges of some representative animal viruses are given, quoted from a selection by Rivers (104).

Bacteriophage. Kligler and his co-workers (70), using a so-called " protein-free " bacteriophage, found that the active agent was amphoteric in acid and decidedly alkaline solutions, and chiefly negatively charged in neutral and mildly alkaline solutions. Krueger and his associates (76) stated that the bacteriophage is negatively charged between the hydrogen-ion concentrations of 9·0–3·4, and positively charged at pH 3·35. Todd (125) found that the active agent carried a negative charge between the hydrogen-ion concentrations of 3·36–7·6.

Rabic Virus. According to Glusman (49) and his associates fixed rabic virus is negatively charged over a range of pH 6·0–9·3.

Vaccine Virus. Douglas and Smith (30) found that vaccine virus carried a negative charge between the hydrogen-ion concentrations of 5·5–8·4.

Fowl-pox Virus. Kligler and his co-workers (70) found fowl-pox virus in " protein-free " preparations to be positively charged on the acid side, amphoteric in neutral solutions and negatively charged in alkaline solutions.

Foot-and-mouth Disease Virus. Olitsky and Boëz (92) believe that the virus of foot-and-mouth disease is positively charged, while Sichert-Modrow (111) is of the opinion that the active agent carries a negative charge over a range of pH 7·0–8·1.

Poliomyelitic Virus. According to Olitsky, Rhoads and Long (95), poliomyelitic virus wanders to the anode.

Rous Virus. Pulcher (102) found that the Rous virus was adsorbed on electro-positive and not on electro-negative hæmoglobins, and concluded that the active agent is negatively charged.

To the above may be added some results of Hindle and Findlay (58), who find that the virus of yellow fever is negatively charged at pH values 5·2–7·0. Findlay also finds (41) that the virus of fowl-pox is negatively charged at pH values 6·6–8·0, and the agent of Rous sarcoma is negatively charged at pH values 5·6–8·0.

It will be seen from a study of the foregoing data that most workers are agreed that plant and animal viruses migrate to the anode, and many state that these viruses are negatively charged and are thus similar to bacteria in this respect.

The same objection, of course, may be raised to this work as can be brought against experiments on the measurement of particle size, namely, that it is the protein carrier of the virus which is being investigated rather than the virus itself. On this account Rivers (104) considers that, at present, it is impossible to state definitely what electrical charge is carried by the virus particle.

A simple method for determining the electrical charge carried by virus particles has recently been described (Bedson and Bland (14)), and some details of this are given here. It is well known that if strips of filter paper are dipped into aqueous solutions of eosin and methylene blue, the eosin will travel up the paper a considerable way, while the methylene blue never rises more than a centimetre above the stain level. This phenomenon is due to the fact that the paper is negatively charged and the methylene-blue particles positively charged, leading to a firm adsorption of the stain on the paper, and thus preventing it being carried along with the water. Eosin, being negatively charged, is not prevented from travelling into the paper. Bedson and Bland have made use of this phenomenon in determining the electrical charge of virus particles in the following way. Strips of blotting paper, 0·5 cm. broad and some 18·0 cm. long, were cut out, and, starting 0·5 cm. from one end, were marked in pencil with a centimetre scale up to 10 cm. The virus, suitably diluted with the requisite buffer, is placed in a glass receptacle—watchglass or short wide-mouthed tube—which stands on the base of a burette stand. A strip of blotting paper, held at one end in the clamp on the stand, is carefully lowered so that the free end dips into the virus to the extent of 0·5 cm. The fluid is allowed to rise up to the 10 cm. mark, the strip withdrawn and the wetted portion cut into sample lengths, each one being dropped into a numbered sterile tube. The first centimetre above the virus need not be tested as the virus will certainly be there, whatever the charge, and it is unnecessary to test the last 3 cm. of the wetted strip because even eosin fails to penetrate as far as this under the conditions of the experiment. The presence of virus in the centimetre lengths 2–6 is then tested for by inoculation.

Bedson and Bland point out that in the case of methylene blue and eosin, where the particles are strongly charged, the stain either passes into the paper or it does not. In the case, however, of a virus at a given *p*H one might be close to, but on the acid side of, its isoelectric point. Here the particles would be weakly positively charged, and their behaviour would be something between that of eosin and methylene blue ; a number of readings over a range of *p*H would therefore be required, and the range chosen has been *p*H 6·6, 7·0, 7·6 and 8·0. On the alkaline side of the isoelectric point similar readings should be obtained whatever the *p*H, but on the acid side the virus particles should pass less and less farther into the paper as the reaction becomes more acid.

To test this an experiment was performed with hæmoglobin, the isoelectric point of which is known, and the results obtained supported this line of reasoning.

By using the above method Bedson and Bland found that the virus of vaccinia is negatively charged over a range of *p*H 6·6–8·0, and this agrees with the results of the cataphoresis experiments of Douglas and Smith (30).

Herpes virus was found to be negatively charged at *p*H 7·6 and 8, and positively charged at *p*H 7·2, 7·0 and 6·6. This is in agreement with the findings of Natarajan and Hyde (89A), who found the virus to be negatively charged only between the hydrogen-ion concentrations of 7·0–8·9.

The writer has applied this method in some studies on the electrical charge of the potato viruses, X and Y, and over a range of *p*H 4·6–7·0, both viruses mounted the blotting-paper strip as far as the sixth centimetre mark, thus indicating a negative charge.

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CHAPTER V

INSECTS IN RELATION TO VIRUSES

Survey of the insect species concerned in virus transmission—Numerical analysis of insect types in relation to virus transmission—Technique of insect management for plant virus studies.

A Survey of the Insects concerned in Virus Transmission

A LIST of the insect orders is given, classified according to Imms (22); those orders concerned in plant virus transmission are marked in heavy type.

SUB-CLASS I. APTERYGOTA.

Order 1. Thysanura.
2. Protura.
3. Collembola.

SUB-CLASS II. PTERYGOTA.

DIVISION I. EXOPTERYGOTA

Order 4. **Orthoptera.**
5. *Dermaptera.*
6. *Plecoptera.*
7. *Isoptera.*
8. *Embioptera.*
9. *Psocoptera.*
10. *Anoplura.*
11. *Ephemeroptera.*
12. *Odonata.*
13. **Thysanoptera.**
14. **Hemiptera.**

DIVISION II. ENDOPTERYGOTA

Order 15. *Neuroptera.*
16. *Mecoptera.*
17. *Trichoptera.*
18. *Lepidoptera.*
19. **Coleoptera.**
20. *Strepsiptera.*
21. *Hymenoptera.*
22. *Diptera.*
23. *Aphaniptera.*

Those orders of insects concerned with plant virus transmission will now be examined in more detail, and the various viruses with which they are concerned will be indicated.

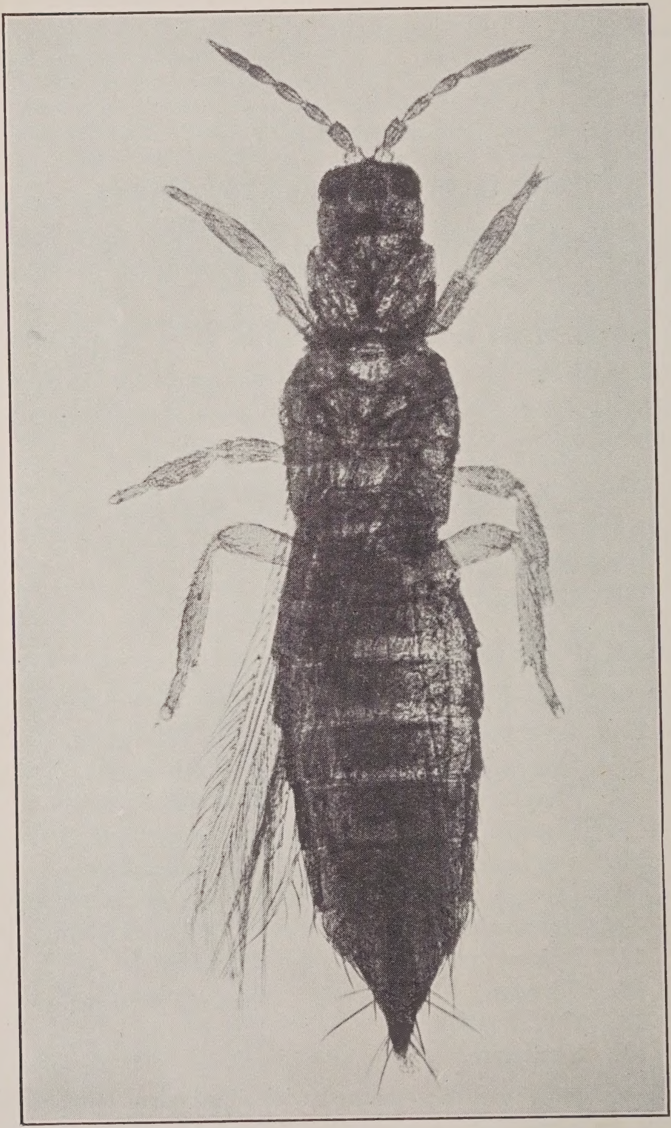

FIG. 11.—*Thrips tabaci* Lind., the insect vector, in Europe, of the virus causing spotted wilt of tomatoes. Adult female (× 140).

Orthoptera. Cockroaches, grasshoppers, etc. Insects with typical biting mouthparts.

There is only one record in the literature of the subject of virus transmission by the Orthoptera: Goss (19) states that the potato virus diseases known as " spindle tuber " and " unmottled curly dwarf " are disseminated by grasshoppers (Locustidæ), *Melanoplus* spp., *M. femur-rubrum*, *M. bivittatus*, *M. plumbeus*, *M. augustipennis*.

Thysanoptera. Thrips. Small or minute slender-bodied insects with asymmetrical piercing mouthparts (see Fig. 11).

Two species of thrips are known to be concerned in plant virus transmission, while others have been doubtfully incriminated. The first of these is *Frankliniella insularis* Franklin, the Black Carnation Thrips, which is the chief vector of the virus of tomato spotted wilt in Australia (36) (see Figs. 12, 13). The other species is *Thrips tabaci* Lind., the Onion Thrips (see Figs. 14, 15). This insect transmits the virus of spotted wilt of tomato in England (41) as well as in Australia. It is also a vector of a virus disease of the pineapple in Hawaii, known as pineapple " yellow spot " (27). There is, however, a possibility that tomato spotted wilt and pineapple yellow spot are caused by the same virus. Moore, in S. Africa (29), describes a virus disease of tobacco transmitted by *T. tabaci*, but this also may be the same virus as that causing spotted wilt in tomato. In addition to the above, there are four doubtful records where the connection between thrips and virus has not been definitely established. These are (*a*) bean mosaic and *Thrips flavus* (5); (*b*) *Capsicum* mosaic and *Thrips* sp. (48); (*c*) Spinach mosaic and *Thrips tabaci* (37); (*d*) sugar-cane mosaic and *Thrips minuta*, var. *puttemansi* (34).

Hemiptera. Plant bugs, leaf hoppers, aphides, whiteflies, etc. Mouthparts piercing and suctorial, the labium in the form of a dorsally grooved sheath receiving two pairs of bristle-like stylets (see Fig. 27).

As will be shown later, the insects comprising the order Hemiptera are more closely concerned with the transmission of plant virus diseases than all the rest of the insect kingdom. It is necessary, therefore, to examine in rather more detail the various subdivisions of this order and the insect families which appear to have an affinity with this class of pathogenic agent. The Hemiptera are divided into two sub-orders, the first of which, the Heteroptera, contains a number of families, two only of which have a slight connection with virus transmission. The second sub-order, the Homoptera, contains about 90 per cent. of the insects concerned all over the world in the dissemination of plant

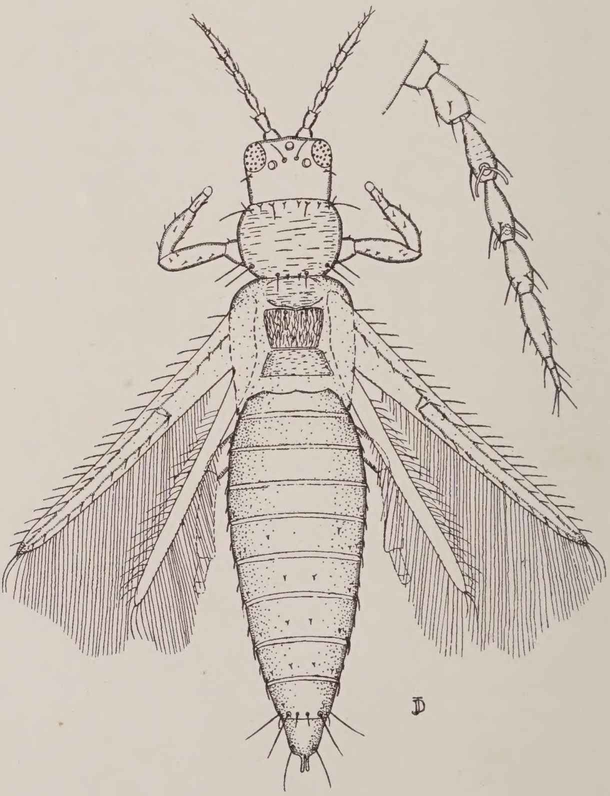

FIG. 12.—The Thrips, *Frankliniella insularis* Frank., the insect vector, in Australia, of the virus causing spotted wilt of tomatoes. Adult male, with antenna further enlarged. *Nat. size*, length about 1·0 mm., width of thorax 0·2 mm. (After Davidson.)

viruses, and these insects are unevenly distributed among five of the dozen or so families which make up the sub-order Homoptera.

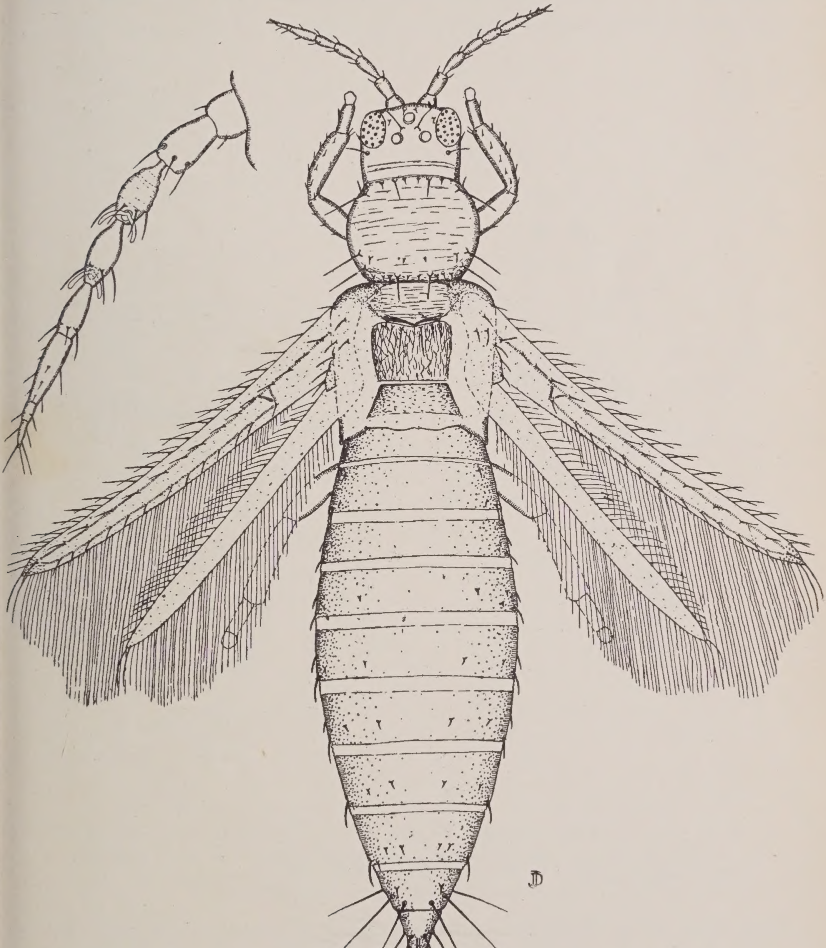

FIG. 13.—The Thrips, *Frankliniella insularis* Frank., the insect vector, in Australia, of the virus causing spotted wilt of tomatoes. Adult female, with antenna further enlarged. *Nat. size*, length, 1·3 mm., width of mesothorax 0·25 mm. (After Davidson.)

Heteroptera. The two families in this sub-order concerned with plant virus transmission are the *Tingidæ* or Lace bugs and the *Capsidæ* (= *Miridæ*) or Capsid bugs. Wille (49) has shown that the insect vector of a serious virus disease of the sugar beet in Germany is a Tingid bug, *Zosmenus* (*Piesma*) *quadratus* Fieb. This disease is known as leaf curl or leaf crinkle of sugar beet, and is characterised by swelling, crookedness and glassiness of the leaf veins and stems (see p. 383). As regards the *Capsidæ*, these bugs have been associated by a number of different workers with various plant viruses, but evidence incriminating these

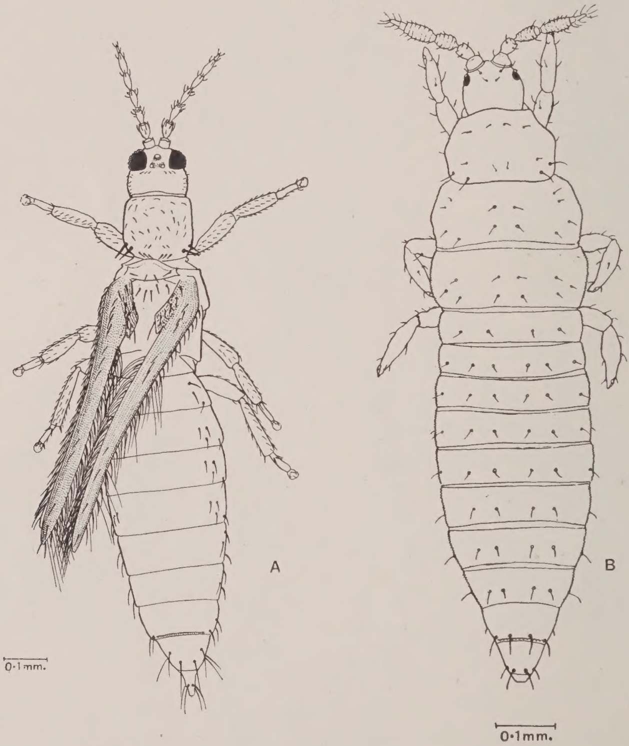

FIG. 14.—A. Adult female, *Thrips tabaci* Lind. B. Second stage larva, *Thrips tabaci* Lind. Drawings to show general arrangement of setæ.

insects is in most cases poorly documented. Goss (19) finds that the virus of potato spindle tuber is transmitted by *Lygus pratensis*, and Elze (12) thinks that the same insect can disseminate the virus of potato mosaic. *L. pratensis* is also stated to be the

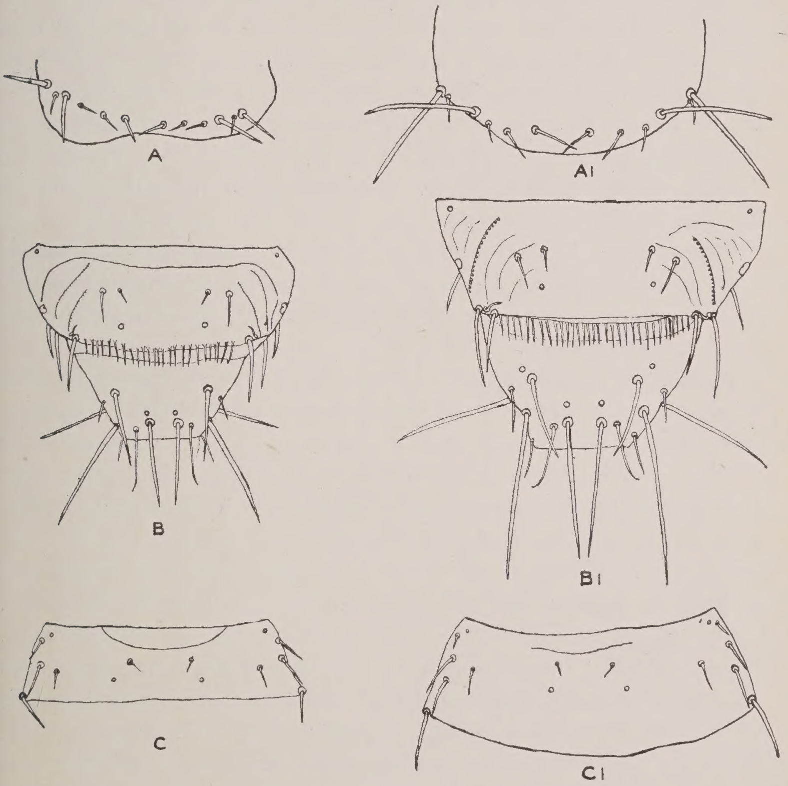

FIG. 15.—Diagnostic characters of *Thrips tabaci* Lind., female imago (A–C) and of *Thrips flavus* Schrank, female imago (A1–C1). × 350.

A and A1. Setæ at posterior margin of prothorax.
B and B1. Chætotaxy of eighth and ninth abdominal tergites.
C and C1. Chætotaxy of second abdominal tergite.
In A1, note greater length of setæ at lateral corner.
In B, note absence of anterior pair of seta spikes on ninth abdominal tergite.
In C1, note presence of four setæ along lateral margins (only three in C). (After Speyer.)

vector of spinach blight (28). It has been suggested (31) that *Calocoris norvegicus* (*bipunctatus*), the potato capsid, can occasionally transmit the virus of potato leaf roll, and that *Calocoris fulvomaculatus* transmits the "squirt" mosaic of hops (3). Novinenko (32) also states that *Orthotylus flavosparsus* is the vector of sugar beet mosaic. In most of these cases further confirmation is needed. It is an interesting point that the Capsidæ, which constitute one of the largest groups of plant-feeding insects, are as a class so little concerned with the dissemination of plant viruses. This point is further discussed on p. 160 in the section dealing with the toxicity to the plant of the insect's saliva in relation to virus transmission.

Homoptera. The families of this sub-order which contain insects associated with plant virus transmission are as follows: *Jassidæ*, *Fulgoridæ*, *Aleyrodidæ*, *Aphididæ* and *Coccidæ*, and these will be dealt with seriatim.

Jassidæ (*Cicadellidæ*) leaf hoppers. These are slender insects, usually tapering posteriorly, which readily jump away if alarmed. After the *Aphididæ* this group is the most closely concerned with plant virus diseases. *Cicadula sexnotata* Fall., the six-spotted leaf hopper, is the specific vector of aster yellows (25), and is also considered to transmit the virus of "yellow dwarf" of onions (10). *Cicadula divisa* Uhl. transmits yellows of parsley and parsnips, apparently a strain of the aster yellows virus (38). *Cicadula mbila* Naude is the specific vector of streak of maize (42), *Eutettix tenellus* Baker transmits curly top of sugar beet in North America (6), and *Agallia sticticollis* Stål. transmits what appears to be the same virus in the Argentine (14). Cranberry "false blossom" is transmitted by *Euscelis striatulus* Fall. (9), and mosaic of rice by *Nephotettix apicalis* Motsch. *Macropsis trimaculata* Fitch has been recently shown by Kunkel (26) to be the vector of the virus of peach yellows.

In addition to the foregoing, there are records of other viruses thought to be disseminated by leaf hoppers, but further confirmation is required in these cases. They are as follows: bean mosaic transmitted by *Typhlocyba picta* and *Chlorita solani*; peanut rosette by *Cicadulina arachidis*, *C. similis* and *Empoasca* sp.; potato leaf roll by Typhlocyba sp., *Eupteryx auratus* and *Empoasca fabæ;* sugar beet mosaic by *Euscelis striatulus*, *Cicadula sexnotata* and *Empoasca flavescens*.

Fulgoridæ. Lantern flies.

Peregrinus maidis is thought to be the vector, in Hawaii, of that important virus disease mosaic of sugar cane; it also transmits mosaic of maize (24).

Aleyrodidæ. Whiteflies. Very small moth-like insects with wings whitish or clouded, body and wings more or less mealy.

A serious virus disease of cotton in the Sudan, known as leaf crinkle, is transmitted by a species of Aleyrodes, *Bemisia gossypiperda*. The same insect transmits a somewhat similar disease affecting the tobacco plant, *i.e.*, tobacco leaf curl (45), while Thung (47) considers that apparently the same disease of tobacco, known in Java as "kroepoek," is there transmitted by another, as yet unidentified, species of Aleyrodes. According to Kufferath and Ghesquière (23) the virus of cassava mosaic is carried by a new species of Aleyrodidæ, *Bemisia mosaicivecta* n.sp., and also by sap inoculation.

Aphididæ. Greenfly, plant lice.

The aphides are concerned with the transmission of more plant virus diseases than any of the other insects, and appear to have a special affinity for the spread of this class of disease. Certain species of aphides, such as *Myzus persicæ* and *Macrosiphum gei*, show a greater capacity in this direction than other species in the group. The following is a list of the aphis species and the virus diseases with which each is associated. A bracket round the name of a virus disease indicates that its association with the aphis is not, in the writer's opinion, definitely authentic.

Myzus persicæ Sulz. Potato aphis of Great Britain; peach or spinach aphis of U.S.A. (see Fig. 16).

Potato virus Y (= Hamilton's Hyo II).
Potato leaf roll.
Potato spindle tuber.
Mosaic of crucifers, mustard, cabbage, turnip.
Mosaic of beans.
Mosaic of the vine.

Mosaic of cucurbits.
Mosaic of celery.
Mosaic of lettuce.
Mosaic of tobacco on certain hosts.

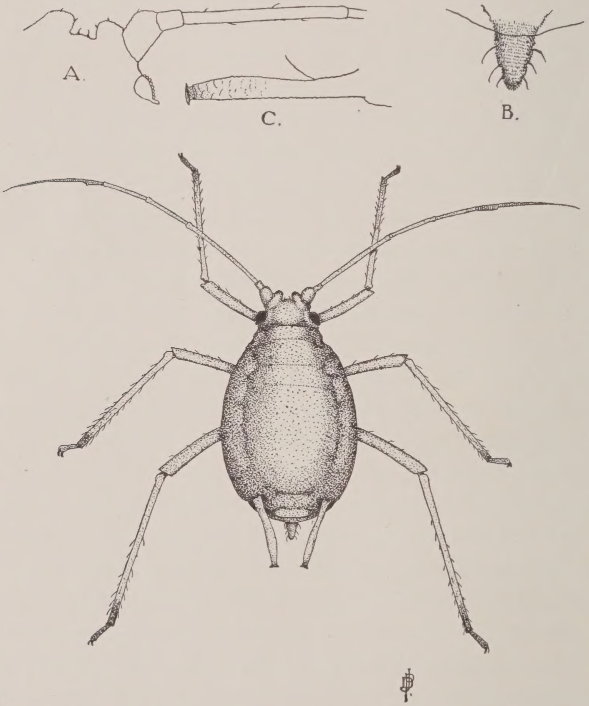

Fig. 16.—*Myzus persicæ* Sulz. Apterous viviparous female. × 20. A, head and antenna; B, cauda; C, cornicle. × 45. Note slight swelling of the cornicles. This insect is concerned in different parts of the world with the spread of thirteen plant viruses and is the chief vector of the viruses affecting the potato.

Mosaic of sugar beet.
Mosaic of dahlias.
Spinach blight.[1]
" Break " of tulip.

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[1] Now shown to be due to the same virus as that causing cucumber mosaic (Hoggan) (21).

Myzus fragæfolii.

Yellows of strawberry.

Witch's broom of strawberry.

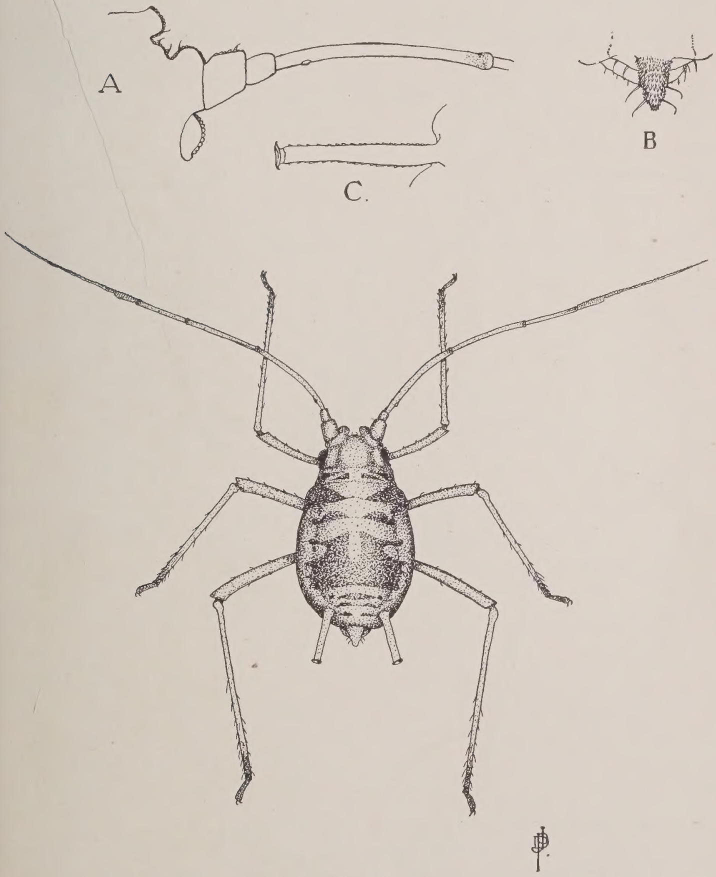

Fig. 17.—*Myzus circumflexus* Buckt. Apterous viviparous female. × 27. A, head and antenna; B, cauda; C, cornicle. × 60. This aphis is an occasional vector of potato leaf roll.

Myzus circumflexus. (See Fig. 17.)

Potato leaf roll.

Myzus pseudosolani. (See Fig. 18.)
Potato leaf roll.
(Potato mosaic.)

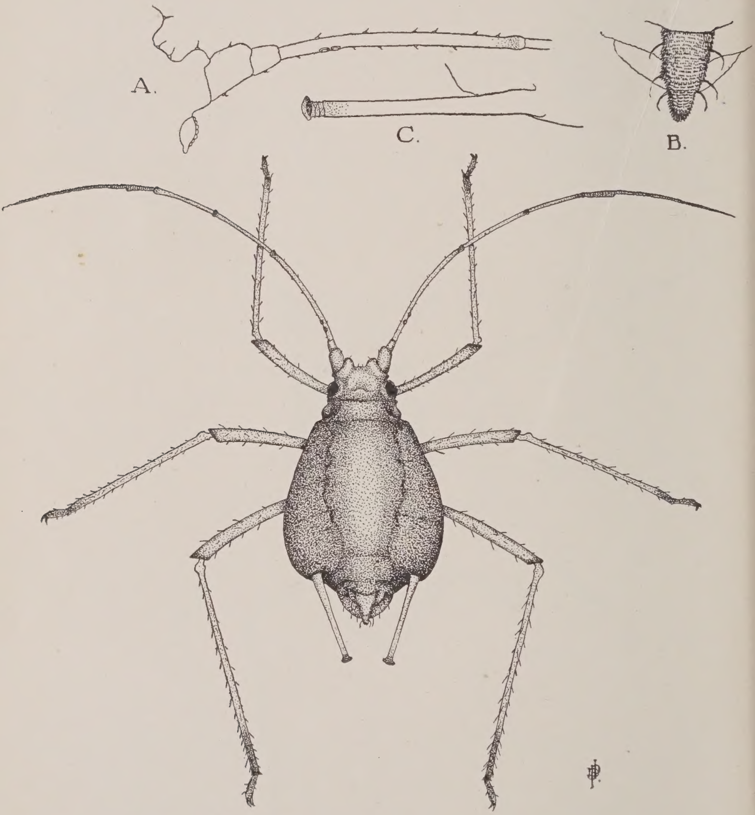

FIG. 18.—*Myzus pseudosolani* Theob. Apterous viviparous female. × 20. A, head and antenna; B, cauda; C, cornicle. × 45. Note cylindrical cornicles. This aphis is a vector of potato leaf roll.

Myzus hieracii.
Mosaic of lettuce.

Myzus pelargonii.
" Break " of tulip.

Macrosiphum gei (*solanifolii*). (See Fig. 19.)
Mosaic of beans.
Mosaic of cucurbits.

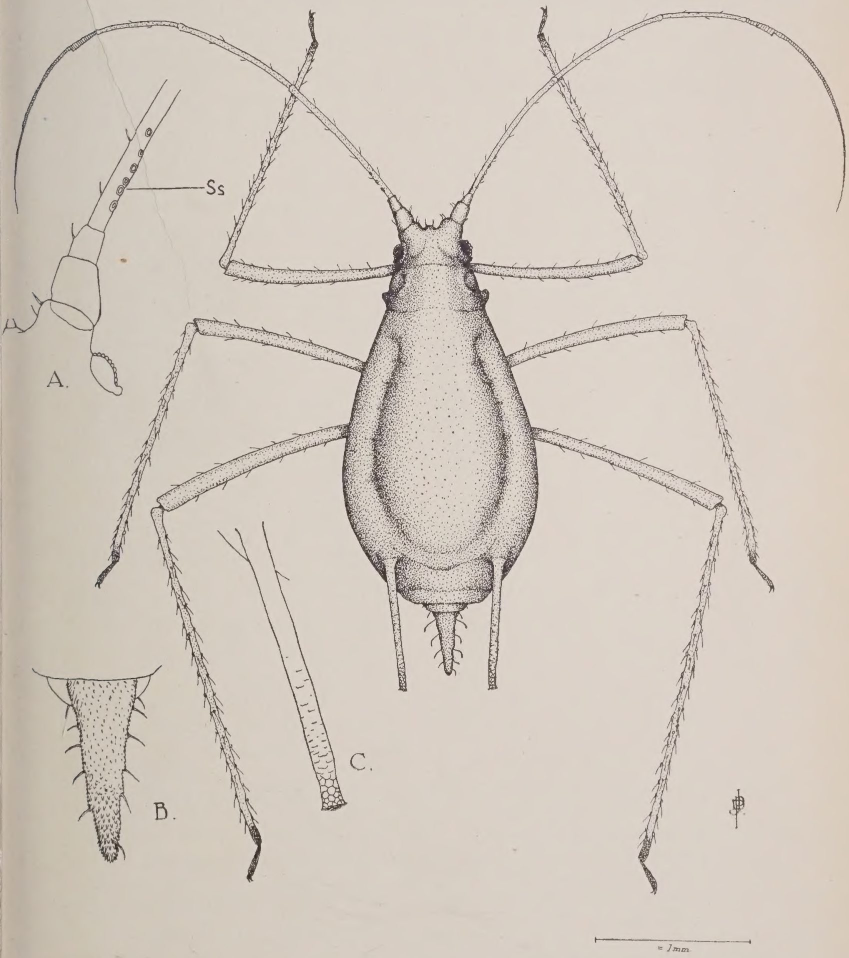

FIG. 19.—*Macrosiphum gei* Koch (= *solanifolii* Ashm.). Apterous viviparous female. × 20. A, head and antenna; B, cauda; C, cornicle. × 45. Ss, sensoria. This insect is concerned in the transmission of five or six plant viruses, but its connection with the virus diseases of the potato is problematical.

"Break" of tulip.
Potato spindle tuber.
(Potato mosaic.)
Spinach blight (= cucumber mosaic).
Tobacco mosaic from tomato.

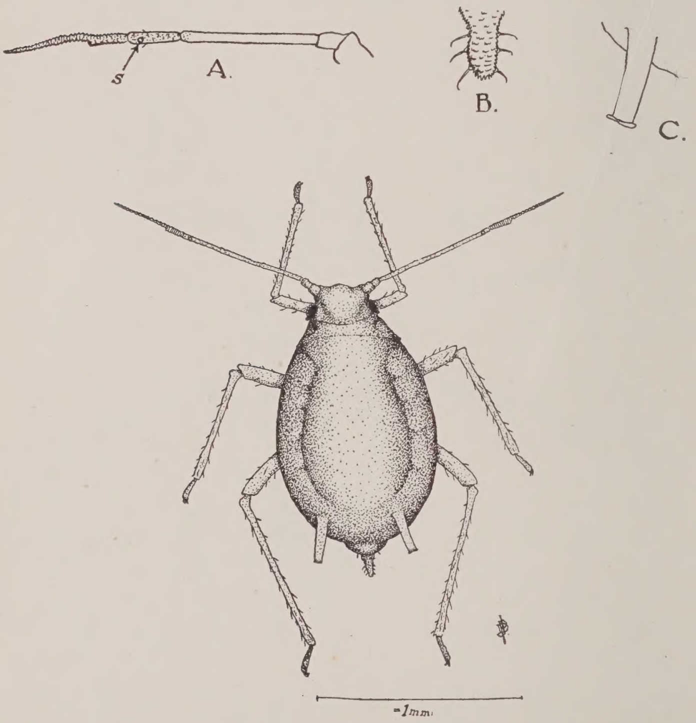

Fig. 20.—*Aphis rhamni* Boyer (= *A. solanina* Pass. and *A. abbreviata* Patch). Apterous viviparous female. × 27. A, antenna; B, cauda; C, cornicle. × 60. *S*, sensorium. Note that this sensorium is usually found on the fifth antennal segment.

This aphis has been stated to be a vector of certain potato viruses, but its connection with their dissemination is doubtful.

Macrosiphum pisi.

Spinach blight (= cucumber mosaic).
Mosaic of peas.
Mosaic of red clover.

Macrosiphum tabaci. = gei (KOCH; SOLANIFOLI ASHM
(Mosaic of tobacco.)

Macrosiphum ambrosia.
Mosaic of beans (50).

Aphis rhamni. (See Fig. 20.) (ABBREVIATA ~~B~~ PATCH)
Mosaic of white clover.
Mosaic of *Cirsium arvense.*
(Mosaic of potato.)
(Leaf roll of potato.)

Aphis rumicis.
Mosaic of beans.
(Leaf roll of potato.)
Mosaic of sugar beet.
Spinach blight (= cucumber mosaic).
Yellow dwarf of onions.

Aphis leguminosæ Theo.
" Rosette " of peanuts.

Aphis gossypii.
Mosaic of beans.
Mosaic of cucurbits.
(Mosaic of Gladiolus.)
Yellow flat or rosette of the lily.

Aphis maidis.
Mosaic of sugar cane.
Yellow dwarf of onions.

Aphis rubiphila.
Mosaic and leaf curl of raspberry.

Aphis idæi.
Mosaic and leaf curl of raspberry.

Aphis laburni.
(Rosette of ground-nuts.)

Amphorophora rubi.
Mosaic and leaf curl of raspberry.

Brevicoryne brassicæ.
Mosaic of crucifers.
Mosaic of beans.

Pentalonia nigronervosa.

Bunchy top of banana and manila hemp.

Hyalopterus atriplicis.

Mosaic of beans (50).

Coccidæ. Scale insects, mealy bugs.

There seem to be few authentic records of transmission of viruses by coccids. Fajardo (13) considers that an undetermined species of mealy bug can disseminate bean mosaic, and Elmer (11) is of opinion that the same virus is transmitted by the mealy bug *Pseudococcus maritimus* Ehr., while Olitsky (33) states that *Pseudococcus citri* is the vector of tobacco and tomato mosaic. A virus affecting the cactus *Epiphyllum truncatum* is said to be transmitted by *Orthezia insignis* (4).

An interesting case of a systemic disease induced in pineapples by a toxin injected by the mealy bug *Pseudococcus brevipes* is dealt with on p. 168.

Coleoptera. Beetles. Mouthparts adapted for biting.

There are few authentic cases of plant virus transmission by beetles. The following records are worthy of mention, but for the moment they should be accepted with reserve :—

Ceratoma trifurcata F. Cowpea mosaic.

Diabrotria vittata	Cucurbit mosaic.
D. 12-punctata	
Epitrix cucumeris	Potato spindle tuber.
Systena elongata	
Discenycha triangularis	
Leptinotarsa decemlineata	
Psylliodes affinis	Potato leaf roll. Potato mosaic.

Numerical Analysis

The following numerical analysis of the insect types and their association with plant viruses reveals the very unequal distribution among the various insect groups of ability to transmit plant viruses. With regard to biting insects, the Orthoptera are concerned with the transmission of one virus only, while the

Coleoptera are thought to disseminate three viruses, with two other records which need further confirmation. All the rest of the known plant viruses are disseminated by sucking insects. The numerical relationships among this latter group are as follows : in considering the Thysanoptera, it is found that there are two viruses known to be transmitted by thrips, with four others where the connection is not definitely established.

Then as regards the Hemiptera-Heteroptera, there is one case in this group of virus dissemination by a Tingid bug and two by Capsid bugs with three doubtful cases in regard to the latter. In the Hemiptera-Homoptera, leaf hoppers, including a Fulgorid, are responsible for virus dissemination in eight instances, with four cases which require further confirmation. In the Aleyrodidæ whiteflies transmit viruses in two cases, and in the Coccidæ there are two or three rather doubtful instances. Then, in considering the Aphididæ, these insects are found to be responsible for the transmission of no less than twenty-seven plant viruses in which twenty-two species of aphides are concerned. It is clear, therefore, that the Aphididæ are the most efficient insect vectors of plant viruses, and no other group of insects approaches them in this respect. One species of aphis seems to have a decided affinity for the dissemination of virus diseases ; this is *Myzus persicæ* Sulz., which is associated with thirteen separate plant viruses. It is possible that this apparent affinity may be partly accounted for by the omnivorous habits and cosmopolitan character of the aphis, but in the writer's opinion there are probably other reasons as well.

Another aphis species, *Macrosiphum gei* Koch, is also associated with the spread of more than one virus ; in this case six in number. Next in efficiency of virus transmission come the Jassidæ (including the Fulgoridæ) with eight vectors. It is of interest to find that the Capsidæ, which constitute a very numerous group of plant-sucking insects, should be so little concerned with plant virus transmission. This point is further considered in the section dealing with the toxicity to the plant of the saliva of insects (see p. 160).

The virus diseases affecting animals are not nearly so closely concerned with insect vectors as are the similar affections of plants. Nevertheless, certain animal viruses are associated with insects, and it will be of interest briefly to compare this association with the parallel phenomenon in plant viruses. As would perhaps be expected, the vectors of animal viruses are confined to the blood-sucking arthropods. There seems to be in some cases the same absolute species-specificity as with vectors of plant viruses, while in others there may be more than one insect species capable of transmitting the same virus. The following insects and other arthropods (ticks and mites) are concerned in the transmission of animal viruses : *Pediculus corporis*, the body louse, is instrumental in spreading the infective agent of trench fever. It is not certain that the bites of this insect alone are sufficient to spread the disease, but absorption of virus from excreta or crushed lice through scratches is thought by British workers to have been the common route of infection. The American workers, however, consider the bite of the infective insect alone to be sufficient to transmit the disease (46). *Pediculus corporis* is also instrumental in disseminating the virus of the rickettsia disease, typhus. Here, again, it is not certain whether it is the bite of the infective insect which transmits the disease, but Bacot and Arkwright (1) concluded that infection was due to contamination of a scratch by lice excreta. They were of the opinion that infection was not conveyed by the actual bite of the louse. Arkwright states (46, p. 399) : " The overwhelming experience during the war from many sides has made the louse theory of transmission to be universally accepted, and has shown that, at any rate under the conditions hitherto studied, *epidemic* typhus is dependent on lice for its propagation. The means by which typhus, or typhus-like disease, are transmitted when lice are absent requires further investigation "—a parallel with many plant viruses. *Pediculus corporis* appears to have an absolute specificity for the viruses of typhus and trench fever. There seems to be no specificity of vector in the case of another rickettsia disease known as Mexican typhus fever. Here, according to Mooser and Castaneda (30), the virus multiplies in two species of lice, *Pediculus humanus* and *Polyplax spinulosus*, and in five species of fleas, *Xenopsylla cheopis*, *Cerato-*

phyllus fasciatus, *Leptopsylla musculi*, *Ctenocephalus canis* and *C. felis*.

Blood-sucking Diptera are concerned in the transmission of several viruses. Yellow fever is usually transmitted by the mosquito *Stegomyia fasciata* (= *Aëdes argentius* : *A. ægypti*), but it has been recently shown that other species of mosquito are capable of disseminating infection—*e.g.*, *Aëdes luteocephalus*, *A. apico-annulatus* and *Eretmopodites chrysogaster* (46). *Stegomyia fasciata* is also the chief vector of another disease of the yellow-fever group known as dengue fever. The *Psychodidæ* or sandflies are concerned in the spread of Pappataci or sandfly fever. This virus is carried by four species of *Phlebotomus*—*P. papatasi*, *P. perniciosus*, *P. minutus* and *P. sergenti*.

As regards transmission of viruses by other arthropods—Rocky Mountain spotted fever, a disease of the typhus group, is spread by a species of tick (Arachnida) *Dermacentor venustus*. This is one of the few cases known where a virus is inherited by the progeny of an infective vector (see Chapter VI., p. 148). Rabbits carry the virus of this disease, and it is disseminated among them by the rabbit tick, *Hæmaphysalis leporis palustris*.

The "bont tick," *Amblyomma hebræum*, is the vector of "heart-water," a virus disease affecting sheep, goats and cattle in South Africa (46). Finally, another disease of the typhus group affecting man, Tsutsugamushi, is transmitted by the mite *Trombicula akamushi*. The relationship of this arachnid with the virus is curious and interesting. The larval mites feed only once. When they have fed on an infected host they do not carry the infection to a fresh animal, but while they go through their metamorphosis as nymphs and adults they still contain the virus. The adults and nymphs are vegetable feeders and do not attack animals, but they pass on the virus to the next generation of larvæ, and these transmit it to their hosts (46 p. 437).

From this brief survey of the insect vectors of animal viruses it will be seen that the body lice, *Pediculus* spp., are concerned with the transmission of three viruses and rickettsiæ: typhus, Mexican typhus, and trench fever; the Diptera with three viruses: yellow fever, sandfly fever and dengue fever; while various species of fleas (*Aphaniptera*) are associated with Mexican typus fever. As regards transmission by the *Arachnidæ*, ticks are the vectors of two disease agents—Rocky Mountain spotted fever, and " heartwater "—while a species of mite is the vector in its larval stage of the virus known as Tsutsugamushi.

The Technique of Insect Management for Plant Virus Studies

General Procedure. In preparing stock cultures of insects for use in experimental work with plant viruses, an insect cage is needed which fulfils some at least of the following conditions: It should be light and easily moved to allow for watering, it should admit sufficient light to obviate " drawing " of the host plant and it should be reasonably insect-proof. In producing large cultures of aphids (*Myzus persicæ*) the writer has found a rectangular wooden cage fairly satisfactory. This cage is about 18 in. high and 9 in. square, with glass in three sides and fine-mesh muslin at the back and on top. The cage is fitted with a base of wood about 6 in. deep, which is embedded in sand. The chief disadvantages of such a cage lie in its weight and unwieldiness. A better framework is provided by metal supports as designed by Davidson and described by the writer (40)—this consists of a circular framework made of wire with a galvanized iron base, the frame being covered with fine muslin or " cellophane." An improvement on this is described by Hamilton (20) as follows: " Its basis is the usual type of light metal frame, consisting of four upright wires supporting two galvanized iron bands, one and a half inches and three inches deep respectively. A useful size is fourteen inches high by seven inches in diameter, but the dimensions can be modified in accordance with the purpose of the cage. The walls are of cellophane (The Cellophane Co., 7–9 Bird St., London), and it is roofed by fine bolting silk (Dufour Bolting Silk, Henry Simon Ltd., 20 Mount St., London)." Cellophane, which is a cellulose composition, is a great improvement on glass or muslin, being easily worked, light and perfectly transparent. It has, however, one drawback in that it readily absorbs moisture and expands. When thoroughly dried it shrinks considerably and becomes brittle and liable to split. After some weeks in a glasshouse it becomes thickly covered with a brown fungus, when it should be renewed. The metal bands of the frame should be smeared with a water-proof cement made by dissolving cellulose acetate in ethyl acetate, a sheet of cellophane is quickly and loosely wrapped round the framework, leaving a margin for shrinkage. The free edges are then joined by a liberal application of " pear drop " (cellulose) cement, and a circle of bolting silk is gummed round the edges and stretched over the top by an elastic band. It is then finished by a tight strapping of 1-in. insulating tape. The cages stand in large pot-saucers filled with sand, or, if thrips are under study, in water to avoid pupation by the thrips in the sand.

For feeding insects on small seedlings or upon sprouting potato tubers, the writer has used extensively the ordinary glass lamp chimney of the stable or " hurricane " type. These are made with a rim at top and bottom which allows a rubber band to be adjusted to hold in place a cover of fine muslin or cellophane, while the lower rim or flange allows the glass to be pressed into the soil surrounding the plant, thus completing an insect-proof chamber. Incidentally, it may be mentioned here that large numbers of rubber bands can be obtained cheaply and easily by cutting up an old inner tube of a cycle or motor-cycle tyre. Modifications of this type of cage are described by Drake *et al.* (10) working on the insect vectors of yellow dwarf of onions.

Various methods have been described for controlling very small insects during virus inoculation experiments, or for confining small insects to particular areas of a leaf. Storey (43) has elaborated a " small-tube technique " for confining leaf hoppers to single leaves of plants, and this will be briefly described. Glass tubes of approximately 6 mm. internal diameter and 8 cm. length are held in clips in a manner which causes one end, ground square and smooth, to be pressed against the surface of the leaf. The spring clip is constructed from spring steel, soldered to a short length of brass tubing, and the whole is supported by a simple device made from a spring wooden clothes peg and a piece of bamboo (see Figs. 21, 22).

A different type of cage for confining leaf hoppers or similar insects to small areas on leaves or stems of plants has been devised by Floyd Smith (17). A straight-grained board of white pine, 12 in. long, three-fourths of an inch thick and of any convenient width, has a hole seven-sixteenths of an inch in diameter bored through from edge to edge about 4 in. from one end. The board is sawn lengthwise into strips seven thirty-seconds of an inch in thickness. Using a sixteen-gauge circular saw which cuts a three thirty-seconds of an inch kerf, each strip is then sawed through the bored portion and for 8 in. of its length. This splits the strip into halves, each approximately one-sixteenth of an inch in thick-

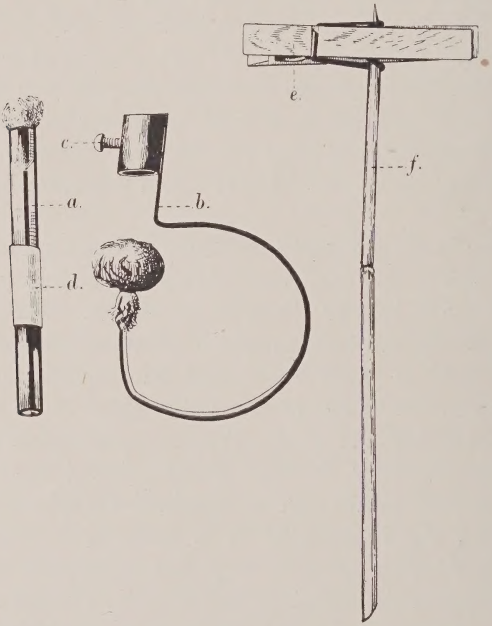

Fig. 21.—Small-tube technique used in transmission experiments with leaf hoppers, showing glass tube (*a*) in which the insect is confined; the spring-clip (*b*) in which the tube is held by the screw (*c*) bedding against a ring of rubber adhesive plaster (*d*); and the supporting stand, made from a spring clothes-peg (*e*) and piece of bamboo (*f*), passing through the middle of the spring. (After Storey.)

ness. The lower 4 in. of the strip is not sawn. These parts, when cut at this thickness, were found to be more resilient than thicker ones and less fragile than thinner ones. To the inner side of one half of the cage over the bored hole is attached a rectangular

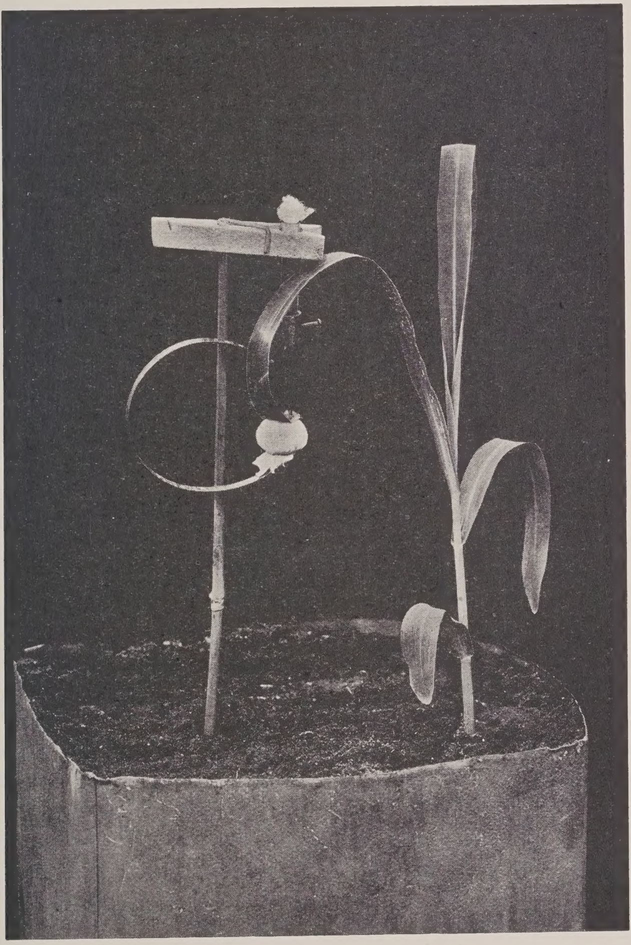

Fig. 22.—The small-tube apparatus, illustrated in Fig. 21, in position upon a maize seedling. (After Storey.)

piece of heavy flannel, which serves as a buffer for the leaf. To the inner side of the opposite half of the cage and centred over the bored hole is attached a 15 mm. glass micro. ring, such as is used for mounting specimens on slides. Sealing-wax was found most suitable for attaching the cell parts to the wood of the cage. The edge of the free end of the ring has attached to it a narrow circular strip of flannel cloth, which thus presents a resilient surface for contact with the leaf. A rectangular piece of silk bolting cloth is attached to the outside of the cage immediately below the bored hole and arranged so that the free end can be drawn across the opening and held in place by two insect pins. When the cage is in use, the two halves of the wooden strip are held together with a rubber band at the top end. By suitable modification the cage can be used to enclose a portion of the stem or petiole (see Fig. 23).

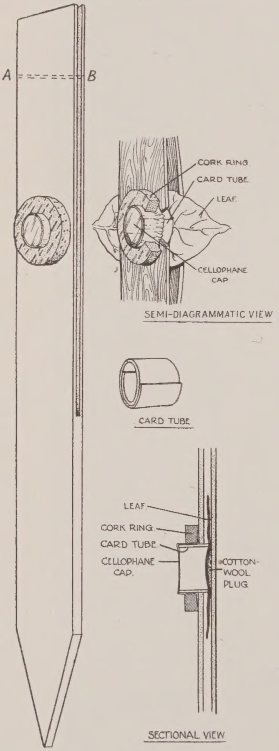

Fig. 23.—A micro-cage devised for controlling small insects such as Thrips in plant virus studies.

The two foregoing types of leaf cage, while suitable for studies with leaf hoppers, cannot easily be used for very small insects such as thrips. For virus studies with these insects the writer (41) has used a modification of Floyd Smith's micro-cage, and this is illustrated and adequately explained in Fig. 23.

Samuel, Bald and Pittman (36) have modified Storey's "small-tube technique" for use with thrips and have found this satisfactory under their conditions. They used a semi-circular piece of steel wire as a spring, soldered to a piece of thick galvanized wire as support, and bearing on its other end a flat piece of cork to which is gummed a piece of sheet sponge-rubber. On the top of the wire support is fixed a spring clothes peg into which a short glass tube can be inserted and so adjusted as to press evenly with moderate pressure against the sponge-rubber stop. The glass tubes used were about 4·5 cm. long and 9 mm. internal diameter, very smoothly ground and preferably of glass at least 1 mm. in thickness.

Another type of leaf cage suitable for thrips transmission studies is described by Sakimura (35). This consists of a piece of thick white felt, three-quarters of an inch square with a three-eights inch diameter hole in the centre, fixed to the leaf with two pieces of celluloid and an ordinary stationery clip. The felt is set on the leaf and the insect transferred into the central hole. A piece of celluloid or stiff cellophane is placed below the leaf and a similar piece over the hole in the felt; the whole is then fastened together with a paper clip. Thrips can be freely transferred to these cages with camel's hair brushes.

Another method suitable for raising stock cultures of thrips but not for making virus inoculations is as follows: Sections of glass tubing of the requisite length and diameter are held upright in a yucca block. A cap of cellophane (No. 300 permeable) or bolting silk is placed over the upper end of the tube. A strip of cotton wool is twisted tightly round the petiole of the leaf to be used for food before it is inserted in the tube, thus securely plugging the lower end. The block of yucca or other material is then set upon a small Petri dish containing water, and the petiole, protruding from the tube below the lower edge of the block, has access to the water. Some of the advantages of this type of cage are that the leaves can be kept fresh and easily changed, the thrips can be readily observed, little space is required and the whole unit is very inexpensive (2).

For transferring or handling leaf hoppers Kunkel (25) describes a useful insect catcher. This is a simple device made as follows: A piece of rubber tubing about 30 in. long and five-sixteenths of an inch in outside diameter is fitted over one end of a small piece of glass tubing 5 in. long and one-fourth of an inch in outside diameter. Two small pieces of cheesecloth are placed over the other end of the small glass tube, making a cap. One end of a second glass tube about 5 in. long and nine thirty-seconds of an inch inside diameter is fitted over the cloth-capped end of the smaller tube. The other end of this tube is heated and drawn out, so as to have an inside diameter of approximately three thirty-seconds of an inch. It is important that the two glass tubes be of such diameter that the one over which the cheesecloth is placed fits easily yet snugly into the other. When catching leaf hoppers the free end of the rubber tube is placed in the mouth. The end of the glass tube having the small opening is placed near the insect to be caught, and air is quickly sucked through the tube. In this way insects are drawn through the small opening on to the cheesecloth. They are blown out by forcing air through the tube in the opposite direction.

Technique of Feeding Insects on Solutions. By feeding specific insect vectors upon suspensions of a virus, it is possible in a limited degree to examine the physical properties of a virus which is insect-borne but is not sap-transmissible. Carter (7) devised a method for feeding the specific vector of curly top of sugar beet, *Eutettix tenellus* Baker, upon a suspension of the virus. He used a thin animal mesentery or " fish skin " in the following manner. No. 4 rubber stoppers were drilled out with as large a drill as could be conveniently used and a small circle of the membrane fastened over the hole in the large end of the stopper, either with shellac or with some reliable water-proof adhesive. This stopper is then fitted into a short tube containing the food solution. A large stopper holds the tube in place in a glass cylinder, which serves as a cage for the insects. For the feeding of a large colony of insects a small sack may be made of the membrane, which is then filled with the solution and hung free in a cage. In a subsequent note, Carter (8) describes an improvement in the technique whereby the fish skin is replaced by a product—probably synthetic—which can be cut to cover one-half of a Petri dish which is completely filled with the solution. The membrane is held in place by a rubber band and care must be taken that good contact is made between the membrane and the surface of the liquid. The Petri dish can be enclosed in any suitable type of cage, and for nymphs and wingless sucking insects it is an improvement on the sack hanging free in the cage. Severin and Swezy (39), using this technique, have shown that the virus of sugar beet curly top will pass through coarse, medium and fine Berkfeld candles.

Storey (44), in proving the filterability of streak disease of maize, which is a non-inoculable virus with a specific insect vector, the leaf hopper *Cicadulina mbila* Naude, used a feeding chamber which was a slight modification of that used by Carter. The fluid is contained in a glass tube, the end of which is covered with a piece of the " Baudruche capping skin " employed by Carter, held in place by a ring cut from a rubber bung suitably bored. This bung fits the end of a glass tube, which forms the insect cage. The rubber bung and membrane form a flush surface, closing the end of the tube completely and leaving no cracks into which hoppers can force their way (see Fig. 24). The apparatus is clamped in a sloping position so that the light from a window shows through the feeding

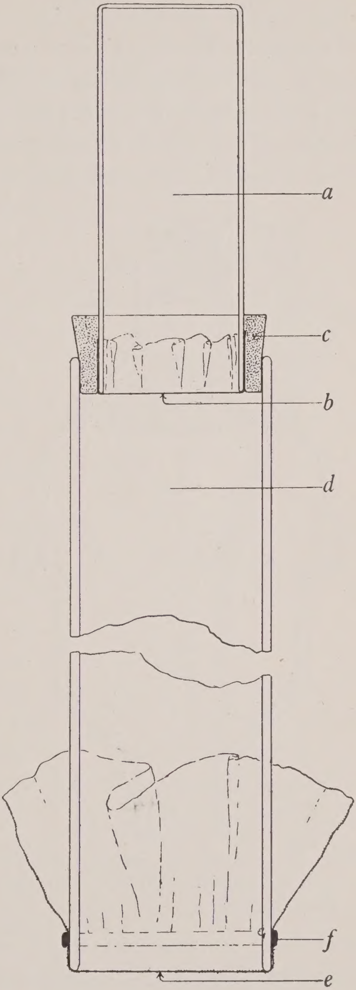

FIG. 24.—A simple apparatus for feeding leaf hoppers on virus solutions. The glass tube *a*, containing the feeding fluid, is closed by the membrane *b*, held in place by the rubber ring *c*. This ring fits the end of the glass tube *d*, 8 in. long × 1 in. diameter, the other end being closed by the muslin *e*, held in place by the rubber band *f*. (After Storey.)

membrane, to which the leaf hoppers consequently move. Hamilton (20) describes a similar technique for feeding aphides upon virus suspensions. This apparatus consists of a pair of cone-shaped glass capsules, the apices of which form wide necks. "A" stands on a ground-in shelf (*s*) in "B" (see Fig. 25). It is covered with fish skin (*m*) and contains the feeding fluid. If fish skin is not available, gut skin can be used instead; it is obtainable at Boots Cash Chemists by special order. By removing the rubber stopper (R) liquid can be changed or replenished without removing the capsule. Capsule B is perforated by four holes of 1 cm. diameter which are covered with small circles of fine organdie muslin to prevent escape of the aphides. In introducing the aphides the apparatus is inverted so that "B" is resting on "A." The insects are placed on the membrane with a fine camel-hair brush through the neck of "B," which is then closed by a cap of muslin or cellophane (*c*). From 50 to 100 aphides can be introduced in this way without escapes. The apparatus is then reversed and held in position by a clamp. A more simple apparatus suitable for smaller numbers of aphides consists of a small crystallizing dish (diameter, 1½ in.) containing a little feeding fluid, closed with a circle of washed fish skin held in place by an elastic band. A filter funnel (diameter, 2 in.) is prepared for its reception by the construction of a small shelf of paraffin wax and the introduction of a few aphides, the top of the stem being plugged with cotton wool. The dish is inverted

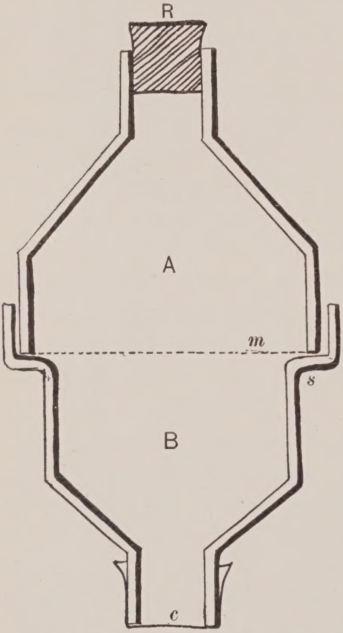

FIG. 25.—Artificial feeding of the aphis, *Myzus persicæ*. (After Hamilton.)

into the funnel and pressed down, and the whole clamped in position (see Fig. 26).

A somewhat different method for feeding insects on solutions has been elaborated by Fife (15), who has substituted a thin section of paraffin wax for the membranes used by Carter. This apparatus was primarily designed for certain bio-chemical investigations where it was necessary to devise a method in which the leaf hopper could be fed artificially on very small amounts of solution of known composition. Pieces of glass tubing 1·5 cm. in diameter and 2 cm. in length served as the cage. One end of the cage was covered with cheesecloth, which was held securely in place by a rubber band. A section of paraffin ribbon, cut 60 microns thick with a microtome, was stuck to the other end of the cage after the leaf hopper had been placed inside. A drop of the feeding fluid was then placed on the top of the paraffin section; this was reached by the insect penetrating the paraffin with its proboscis from the other side. Fife has also designed an apparatus consisting of two small cells on a slide which enables the process of feeding by the leaf hopper to be observed under the microscope.

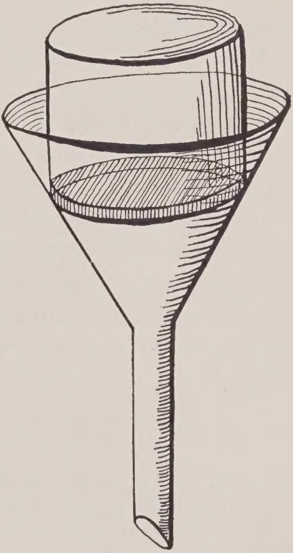

Fig. 26.—Artificial feeding of the aphis, *Myzus persicæ*. Crystallizing dish inverted over filter funnel. (After Hamilton.)

With the foregoing technique the various workers found that it was not possible to feed the insects on the solutions for any great length of time owing to their very high mortality rate. This mortality is probably due to changes taking place in the extracted plant sap, which may thus become toxic to the insect, and also to the development of yeasts and moulds upon fluid exuded through the punctures in the membrane. Carter found that it was not safe to feed leaf hoppers, *Eutettix tenellus*, on solutions for longer than two or three days at 70° C. Storey fed the leaf hopper *C. mbila* for twenty-four hours, while Hamilton did not carry leaf-juice cultures beyond the third or fourth day. The virus fluid used by Storey consisted of the juice of streak-affected maize seedlings, clarified by passage of a sand-and-pulp filter, to which was added an equal quantity of 20 per cent. sucrose solution.

It is clear that by means of this technique for the artificial feeding of insects it is possible to examine some of the physical properties of those plant viruses which are dependent upon a specific insect vector for their transmission and which are not sap-inoculable.

It may also perhaps be possible to use this method to obtain a purer virus suspension than could be produced by precipitation with chemicals or by selective adsorption. Thus, by feeding large quantities of virus-infected insects on a suitable solution it might be possible to obtain a virus suspension in a state of comparative purity.

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CHAPTER VI

INSECTS IN RELATION TO VIRUSES (*Continued*)

Some factors in the relationship between virus and insect vector.

The Evidence for the Existence of an Obligate Relationship between Virus and Insect Vector

THE evidence favouring a specific relationship between certain plant viruses and their insect vectors can best be summed up under the following heads: (*a*) a delay in the development of infective power within the insect, usually described for convenience, though less accurately, as an " incubation period " of the virus in the insect; (*b*) the specificity of an insect for a particular virus; (*c*) selective transmission by an insect from a complex of plant viruses; (*d*) longevity of the virus within the body of the insect vector.

A Delay in the Development of Infective Power within the Insect. There are several records of cases where a specific insect vector of a virus does not become infective to a healthy plant until a minimum time has elapsed after feeding on the diseased plant. These periods vary from four hours to ten days, according to the virus and the insect. Kunkel (18) finds that *Cicadula sexnotata*, the vector of " aster yellows," does not become infective until a minimum period of ten days has elapsed since the first feeding on a " yellowed " plant. Linford (19) states that a similar period of ten days must elapse after feeding before *Thrips tabaci*, the vector of pineapple " yellow spot," can become infective to a healthy plant. Bald and Samuel (1) and the writer (39) have found that periods of seven to five days must elapse after feeding before the thrips which transmits the virus of tomato spotted wilt can become infective. The foregoing periods are the longest delays in the development of infective power which are shown by insect vectors, but there are several cases of similar delays of shorter duration. In the case of *Eutettix tenellus,* the vector of curly top of sugar beet, the waiting period within the insect varies from twenty-one to twenty-four hours (Carsner and Stahl (6)).

Temperature appears to play a part in determining the length of these waiting periods. In streak disease of maize, Storey (41) finds the waiting period to be between twelve and forty-eight hours in the leaf hopper *Cicadulina mbila,* which is the specific vector. These periods are at a temperature of 30°–35° C. with an absolute minimum period of six to twelve hours at 30° C. Elze (11), working with potato leaf roll and the aphis *Myzus persicæ,* considers that there is a delay in the development of infective power within the insect of twenty-four to thirty-eight hours. The writer (38), however, was unable to induce *Myzus persicæ* to infect healthy potato plants with leaf roll before a minimum period of fifty-four hours had elapsed since the first contact of the aphis with the source of leaf roll infection. It is unlikely, however, that these are fixed periods, and they are probably dependent upon temperature and other environmental conditions.

It might be suggested that this so-called incubation period of the virus in the insect merely represents the time taken for this agent to pass down the alimentary canal, diffuse through the gut wall and so return to the saliva, as the group Hemiptera possess an œsophageal valve which prevents any regurgitation of virus-containing sap. Some attempts have been carried out by Swezy (45) and the writer (38) to trace the movement of particles of dye, ingested by the respective insects—leafhopper and aphis—while feeding, through the alimentary canal and so to the salivary glands. It was hoped that it might be possible to correlate the time taken by this movement of the dye particles with the incubation periods of the virus in the insects. The experiments, however, were not successful and it was found in both cases that the stain reached the salivary glands in a very much shorter time than that occupied by the incubation periods of the virus in each insect. It is not certainly known what becomes of the virus after its entrance into the body of the insect vector ;

it is supposed to enter the gut with the imbibed sap and then to pass from the alimentary canal into the blood and finally to arrive in the salivary secretion with which it passes out into the plant host during the process of feeding (see Fig. 27). The route followed by the virus after ingestion by the insect probably influences the insect's subsequent capacity to transmit the virus. At present one must assume that the virus diffuses or migrates through the walls of the gut and so into the blood, whence it makes its way into the salivary glands. A close association of the virus with the salivary glands seems to be a *sine qua non* for transmission by Hemipterous insects.

Some observations by Mooser and Castaneda (23) on the multiplication of the virus of Mexican typhus fever in fleas are of interest in this connection. This disease is of the rickettsia type, and it is possible to observe the supposed organism, *Rickettsia prowazeki*, within the insect vector. Mooser and Castaneda demonstrated this rickettsia in the epithelial cells of the stomach and in the cells of the Malpighian tubules of five species of fleas. Whereas in infected lice enormous numbers of these organisms are discharged from the disintegrating cells into the intestinal content, only a few of the rickettsiæ are found in the lumen of the flea's intestine. This is due to the fact that they are held back by the peritrophic membrane, a membranous tube which lines the stomach, but is not in contact with the epithelium. This peritrophic membrane is absent in the Hemiptera, the group of insects most concerned with plant virus transmission. The rickettsiæ seem to enter the gut almost exclusively by the Malpighian tubules. It is perhaps of interest that no rickettsiæ were found in the salivary glands of the fleas.

Similar delays in the development of infective power within the vector occur in certain cases with animal viruses which are insect-borne. Thus the mosquito, *Stegomyia fasciata*, does not become infective until a period of twelve to fourteen days has elapsed since the first meal on an infective host. The effect of temperature appears to be very marked on this "incubation period." At 22° C. it takes three or four weeks at least for the mosquito to become infective, and at 20° C. the virus is unable to develop. If an infected mosquito, capable of transmitting the disease, is exposed to a low temperature, it ceases to be infective, but recovers this power on again being warmed (46 p. 457). In the case of dengue fever the same mosquito species takes eleven to fourteen days to develop infective power, while the incubation period of the virus of sandfly fever in *Phlebotomus papatasi* is about seven days. Similarly with typhus and trench fever, the louse vector *Pediculus corporis* requires periods of seven to ten days in the first case and five to twelve days in the second to develop infective power.

It is clear, then, from the foregoing examples that in certain plant and animal viruses disseminated by insects there exists what, for want of a better term, may be described as an incubation period of the virus within the insect vector. There is as yet, however, no evidence that this waiting period implies a phase in a developmental cycle of the virus within the insect host. It would be more reasonable to concede the existence of an incubation period of a virus within the insect in those cases where the virus in question can only be transmitted by that particular insect and not by needle inoculation in addition. It is difficult, however, to see any valid reason for an incubation period of a virus in an insect when that virus is also easily transmissible by sap-inoculation from plant to plant. An example of this is given by the sap-transmissible virus of tomato spotted wilt, where the thrips vector has a delay in development of infective power of five to seven days. In this case, however, it may be possible to explain the delay as a necessary multiplication of the virus within the insect before it is capable of transmitting an infective virus dose.

Specificity of Insect Vectors. A rather strong point in favour of a specific relationship between insect and virus lies in the fact that certain plant viruses can be transmitted only by a particular insect species. There are numerous examples of this. Aster yellows can be transmitted only by the leaf hopper *Cicadula sexnotata*, and by no other means except budding. Similarly, streak of maize is disseminated only by the leaf hopper *Cicadulina mbila* and one closely allied species, and curly top of sugar beet in North America only by *Eutettix tenellus*. Other examples of this specificity are shown by leaf curl of cotton and *Bemisia gossypiperda*, and mosaic of sugar cane and *Aphis maidis*.

In addition to the insect *species-specificity* indicated above there is also an insect *group-specificity* shown by certain viruses. Thus the virus of tomato spotted wilt is transmitted by two or more species of thrips, and, so far as is known at present, by no other type of insect. Potato leaf roll is spread by the aphis *Myzus persicæ*, with which it appears to have a special relationship, and also less effectively by two other aphis species, *Myzus pseudosolani* and *Myzus circumflexus*, but not, in the writer's opinion, by insects of other types. The virus of tulip " break " is similarly confined to two or more aphis species. This group-specificity has recently also been commented upon by Storey (42), who remarks how closely a grouping of viruses upon the basis of the type of symptom manifestation would agree with a grouping upon type of insect vector. Most workers recognize the group of mosaic viruses, transmitted for the most part by aphides, and the yellows group, transmitted by Jassids. Storey goes on to say that it is interesting to note that evidence is beginning to reveal a homogeneous group transmitted by whitefly (Aleyrodidæ), where the characteristic symptoms are thickening of the veins and leafy outgrowths on the lower side of the leaf, to which may possibly be added a thrips-transmitted group of viruses whose outstanding symptom is the development of concentric rings.

The specificity shown by certain insects for certain viruses does lend colour to the theory that an obligate relationship exists between them. It does not necessarily imply that the virus is dependent on the insect for a vital change, although, on the other hand, since the virus is not inoculable, it would appear that the insect must attune the virus in some way to enable it to attack the plant host.

It seems to the writer that the crux of the matter lies in the moment of entry of the virus into the plant. It may be that the virus requires specific enzymes supplied by the accompanying saliva of the insect to enable it to start its multiplication within the plant. It is known that the virus will persist for long periods, possibly indefinitely, within the body, probably in the salivary glands, of the insect. Presumably, therefore, the saliva is a suitable medium for the multiplication of the virus, and so would allow of opportunity for an initial multiplication within the plant cell. Such an initial multiplication would break down a possible resistance offered by, or overcome any temporary incompatibility with, the sap of the new host plant. Such an opportunity for readjustment would not be offered in the case of direct transfer of the virus by needle from plant to plant.

It is also quite possible that in a certain environment an insect may be specific for one virus because it fulfils certain necessary local conditions. In another country or district where different environmental conditions prevail, some other insect may be the specific vector. This can be illustrated by the case of curly top of sugar beet. In North America the specific vector of this virus is the leaf hopper *Eutettix tenellus* (6), but in the Argentine the vector of curly top is another leaf hopper, *Agallia sticticollis* (Fawcett (12A)), although it is possible that the two curly top viruses are not identical. Again, in America (28) and Holland (11) the virus of potato leaf roll appears to be disseminated by the aphides *Macrosiphum gei*, *Aphis rumicis* and *Aphis rhamni*, while in England these species have consistently failed to transmit leaf roll (36).

Another point to be considered is the possibility of a plant virus becoming attuned to transmission by an insect not normally a vector. There is at least some evidence that a virus may become attuned to a plant, which normally it does not infect, by passage of certain other plants. It may be that a virus can also be attuned to transmission by an insect by long association with it, especially if the usual insect vector is not indigenous to that particular district as appears to be the case with *Eutettix tenellus* and curly top of beet in the Argentine. This theory is supported by evidence that there exist strains or biotypes of viruses, such as those attacking the Gramineæ for example, which may react differently to insect vectors. In the writer's experience with potato viruses it is no uncommon thing to find that the aphis *M. persicæ* will transmit one virus, while another closely resembling the first is not so transmitted. Further research may reveal the existence of other insect vectors, or transmission under experimental conditions by other insects, in those cases where an absolute affinity between insect and virus is thought to exist at present.

The specificity of insect vectors of animal viruses does not appear to be absolute in many cases, and so does not compare with the degree of specificity of insect vector shown by the plant viruses. The body louse *Pediculus corporis* is thought to be the specific vector of typhus fever, although Arkwright (46 p. 409) speaks of the occurrence of *Rickettsia prowazeki*, the supposed organism of typhus fever in another species of blood-sucking louse, *Pedicinus longiceps*, which occurs on monkeys. *P. corporis* seems, however, to be the sole vector of trench fever. As regards dengue fever, although the mosquito *Stegomyia fasciata* is the most common vector, Morishita (24) has shown that two other species of mosquito, *Stegomyia scutellaris* and *Armigeres obturans*, are also capable of conveying infection.

There is little information on the question of purely mechanical vectors of animal viruses. Howard and Clark (15a) found that the ordinary house fly can carry the virus of poliomyelitis in an active state upon the surface of the body for several days and in the alimentary canal for several hours. The bed bug *Cimex lectularius* was found to ingest the virus with the blood when fed on an infected monkey and to maintain it in viable condition within the body for seven days. The following are mechanical vectors only of the virus of African horse sickness: *Stomoxys calcitrans*, *Lyperosia minuta*, *Tabanus* spp. and *Culicoides* spp. (46 p. 365).

Selective Transmission by an Insect from a Complex of Plant Viruses. Arising as a natural corollary from the specificity of insect vectors for particular viruses is the power of selective transmission exhibited by certain insects when fed on a host plant in which two viruses are co-existing. This phenomenon has been described on more than one occasion and has been used to demonstrate the fact that potato mosaic diseases are usually due to complexes of viruses (37).

If the aphis *Myzus persicæ* be colonized on a tobacco plant infected with the viruses of cucumber and tobacco mosaic respectively and then transferred to healthy tobacco seedlings, these develop only the disease due to the cucumber mosaic virus. The tobacco mosaic virus is not transmitted, although both viruses must have been present in the sap of the infected plant upon which the insects fed. The aphis has therefore selected out the cucumber mosaic virus and left behind the virus of tobacco mosaic (14).

Two other cases of selective insect transmission have been described by the writer. The first of these concerns the potato mosaic group of viruses (37). It has been shown in certain potato diseases of the mosaic crinkle type that aphis transmission from such a diseased potato plant to healthy tobacco gives one disease, while needle inoculation from the same diseased potato, also to healthy tobacco, gives another disease. The explanation of this phenomenon lies in the discovery that the potato plant harbours two viruses, designated X and Y, and while needle inoculation transmits both X and Y, the aphis selects out the Y virus only. The second case of selective transmission concerns some tomato plants affected with a virus disease. These plants were colonized with the larval forms of *Thrips tabaci*, and those were later transferred to healthy tobacco and other plants. Needle inoculation was also made from the same diseased tomatoes to similar healthy plants. The plants colonized with the thrips developed a disease caused by the virus of tomato spotted wilt, while the plants inoculated by the needle developed a different disease, which further investigation proved to be of a composite nature, due to the virus of tomato spotted wilt and another virus, not thrips-transmitted, of the tomato mosaic type (39).

An interesting case of apparent differential transmission by needle and insect respectively is given by some experiments with the virus of cucumber mosaic on tomato. Kraybill and Eckerson (17) by filtration separated the juice from cucumber mosaic tomato plants into two fractions—the residue which contained the mosaic virus and the filtrate which contained a leaf-deforming principle. In a later paper, Kraybill *et al.* (16) have proved that a non-infectious leaf-deforming principle which is heat-stable can be isolated from cucumber mosaic on tomato. The principle is apparently transmissible to young tomato plants by heavy inoculation with preparations obtained from tomato plants affected with cucumber mosaic and rendered non-infectious by filtration. Young tomato plants so infected developed the " fern leaf " or filiform type of leaf deformity and subsequently resumed normal growth. The leaf-deforming principle is apparently not transmissible from plant to plant as is a virus. Mogendorff (22) was unable to produce the " fern leaf " deformity in tomatoes with ordinary tomato mosaic virus ; he could produce it only occasionally by needle inoculation with cucumber mosaic virus, as used by Kraybill *et al.*, but the leaf deformity could be produced regularly and at will by means of aphis (*Myzus persicæ*) transmission of cucumber mosaic to tomato. Apparently the insect picks up or liberates in some way the " non-infectious leaf-deforming principle " contained in the virus of cucumber mosaic, while needle inoculation generally fails to produce the filiform-leaf effect.

In certain cases of selective transmission of plant viruses by insects the host plant itself plays a part in the selection of the virus. Although, as Hoggan (14) has shown, the aphis will select cucumber mosaic out of a virus combination of cucumber and tobacco mosaic in the *tobacco* plant ; in the *tomato* plant the tobacco virus is also aphis-transmissible. Similarly, an experiment carried out by the writer (36) has shown that when the aphis *M. persicæ* is colonized on a potato plant affected with a virus combination of leaf roll and the mosaic virus Y, and then transferred, some to healthy potato and some to tobacco, only leaf roll developed in the potato ; but the development of a mottling disease in the tobacco plant which is immune to leaf roll showed that the aphis was picking up both viruses. Here, then, it appears as if the potato plant was the selective agency.

Longevity of the Virus within the Body of the Insect Vector. The retention by an insect vector of infective power for long periods without again having access to a source of virus infection is fairly strong evidence, if not of specific relationship, at least of multiplication of virus within the body. There are several instances of this prolonged retention of infective power. Kunkel (18) has shown that the leaf hopper *Cicadula sexnotata*, the vector of aster yellows, is capable of retaining the infection after being fed for two months on a host plant immune to this virus. According to Severin (29) infective beet leaf hoppers, *Eutettix tenellus*, retain the infective principle of curly top of sugar beet throughout life. Carsner (6) has kept infective hoppers on immune plants for fifty-eight and one hundred and eleven days without loss of infective power. Smith (34) and Bennett (4) show that the virus of raspberry leaf curl remains active in the body of the vector, *Aphis rubiphila*, for as long as three weeks, and probably for the lifetime of the insect. The thrips *Frankliniella insularis* appears to retain the virus of tomato spotted wilt for at least twenty-four days, including the pupal period (27), while *Cicadulina mbila*, the vector of streak of maize, appears to retain the virus throughout life (41). Elze (11) and the writer (38) have shown that the aphis *Myzus persicæ*, infected with the virus of potato leaf roll, can feed upon immune plants, such as spinach or cabbage, for periods of seven to ten days and then infect healthy potato plants with leaf roll. This aphis is also capable of infecting a number of healthy potato plants with leaf roll without again having access to a leaf roll source (36). McClintock and Smith (21) have shown that aphides can carry the virus of spinach " blight " to five plants over a period of one hundred and twenty hours so far as tested.

In most of the foregoing cases the insect is a specific vector of the virus, or at least appears to have a special affinity for its transmission. There are, however, cases where the infective power is lost by the transmitting insect in a short time, or after having infected a single plant. A good case in point is described by Doolittle and Walker (10), who have shown that a virus-free aphis vector of cucumber mosaic can become infective after only five minutes' feeding on a mosaic plant, and, moreover, can produce infection after feeding on a healthy plant for a like period. The aphis loses its ability to produce the disease immediately after it first feeds on a healthy plant. Infective aphides from mosaic cucumber plants do not transmit the disease after six to eight hours' confinement in a glass tube, this period being approximately the same as that during which expressed cucumber mosaic juice remains active. Similarly, the striped beetle, *Diabrotica vittata*, retains the virus of cucurbit mosaic for a short time only. Here, then, are two fairly definite cases of mechanical transmission. There are also records of individual vectors losing infective power when, as a general rule, they are capable of retaining it for long periods. Storey (41) records that in one definite case the vector of streak of maize lost infective power, while Kunkel (18) found that while some individuals of *Cicadula sexnotata* retained infective power others lost it quickly.

The writer has experimented with the aphis *Myzus persicæ* as a vector of the potato virus Y among healthy tobacco seedlings. The experiments were carried out as follows: Known Y-infective aphides were allowed to feed for twenty-four hours on progressive series of tobacco seedlings, each set of aphids being moved after twenty-four-hour intervals to fresh seedlings without again having access to a source of infection. In two cases only out of fifteen experiments was infection carried to the second set of plants—that is, plants which represented a period of forty-eight hours since the insects had contact with a Y-diseased tobacco plant. In the other experiments infection was confined to the first set, *i.e.*, twenty-four-hour period plants. The aphis, then, appears to lose its infective power after twenty-four hours, and this corresponds fairly closely with the stability of the Y virus in extracted sap, which is inactivated after twenty-four to thirty-six hours' ageing at room temperature. From this it would appear that transmission of the Y virus by *M. persicæ* is purely mechanical, were it not for the fact that this aphis seems to be the only one capable of disseminating the virus, with the possible exception of *Macrosiphum gei*.

A somewhat similar state of affairs exists with the virus of red-raspberry mosaic and the aphis vector *Amphorophora rubi*. This insect quickly loses the virus in short-interval transfers on healthy plants (4).

A survey of the relationship of insects with animal viruses reveals a similar retention of infective power by the vector for long periods. *Stegomyia fasciata* has been found capable of producing yellow fever infection ninety-one days after an infective meal. Siler (32) states that *S. fasciata* carries the virus of dengue fever indefinitely, infection in one case being carried seventy-five days after an infective meal. Similarly, the species of *Phlebotomus*, which transmit sandfly fever, once infected, remain infective all their lives. As regards the virus of typhus fever in the louse, *Pediculus corporis*, it is of interest to find that there is a greater concentration of the virus in the insect than in the blood of an infective host. This presumably indicates a multiplication of the virus within the body of the insect. Weil and Breinl (48) state that while one-hundredth part of a louse emulsion could infect guinea-pigs, it required 3–4 c.c. of infected blood to do so. As already mentioned, Mooser and Castaneda (23) have demonstrated the multiplication of the rickettsia of Mexican typhus fever within the bodies of various species of fleas. The viruses of trench fever and Rocky Mountain spotted fever are also retained, apparently indefinitely, by their respective vectors, the body louse *Pediculus corporis* and the wood tick *Dermacentor venustus*.

Multiplication of the virus of Rocky Mountain spotted fever takes place to a very high degree within this tick, the virus becoming 200–3,000-fold more concentrated. The infective principle is also persistent through the egg into the next generation, the concentration of the virus in the egg being almost as high as in the tick itself (46 p. 444).

In reviewing the facts thus summarized it will be seen that there is sufficient evidence to support the view that a specific relationship exists between insect and virus in certain cases. It is also clear that there exist grades in this relationship which range from absolute specificity, as in the case of aster yellows and *C. sexnotata*, a strong affinity as exemplified by potato leaf roll and *Myzus persicæ* to mere mechanical transmission, as in cucumber mosaic and various aphis species. At the same time there is no evidence that there is any kind of a developmental cycle of the virus within a specific insect vector, although such a possibility cannot be excluded. If an attempt be made to explain some of the foregoing phenomena on purely physico-chemical grounds, the difficulties in the way of such an explanation become obvious. It could be assumed that an insect is specific for a particular virus because this virus can only exist within a narrow range of *p*H, and the particular insect species which transmits it may be the only one which possesses salivary and bodily juices of that particular *p*H. It is, however, unlikely that there exists a big difference in *p*H between the secretions of two allied species of insect both feeding on the same host plant, one of which is the vector of a particular virus and the other is not. Again, one can attempt to explain the " incubation period " of a virus within an insect on a theory of adsorption and eleution. Suppose the ingested virus particles adsorb to the walls of the minute suction canal and of the alimentary canal over a period of days during the first feeding on a source of infection. It could then be assumed that the incubation period represented the time necessary for the gut walls to reach saturation point of adsorption or for the continual flow of ingested sap to commence to eleute the adsorbed particles and that the persistence of infective power of the insect was due to a continual process of detachment of virus particles from the gut walls. Although this speculation might be applicable to insects in which the virus " incubation period " is comparatively short, it could hardly explain those cases where the delay in infective power extends over several days. Finally, the suggestion might be made that the capacity for virus transmission is dependent upon the permeability of the wall of the alimentary canal.

It is perhaps idle to indulge in such speculations until more evidence is forthcoming as to the nature of viruses. There do, however, seem sufficient facts to allow of the assumption that plant viruses multiply, in certain cases only, within the body of the insect vector.

Some Further Points in the Relationship between a Virus and its Insect Vector

Is the Insect Vector of a Virus itself Diseased or Abnormal ? A characteristic of virus diseases affecting both plants and animals is the occurrence of intracellular inclusions, or X-bodies, so-called (see p. 26). These X-bodies, which are apparently peculiar to virus diseases, are considered by a few workers to be the causal organisms of plant virus diseases and to be capable of existing in a filterable form. The general view, however, is that they are a pathological effect of the virus rather than the causal organism itself. The occurrence of these peculiar bodies in the cells of affected plants evokes an inquiry as to whether there exists a parallel state of affairs in the body of an infective insect vector. There appears to be no record of an X-body ever having been found within a virus-infected insect, and this in itself is adverse to the theory that these bodies are the causal organisms, unless it be suggested that these supposed organisms pass into the insect in a filterable phase.

Dobroscky (8) has made an intensive study of the salivary glands and alimentary tract of the leaf hopper, *Cicadula sexnotata*, with a view to finding the causative agent of aster yellows, of which this insect is the specific vector. Dobroscky was unable to find such an agent or any lesion which might be due to the presence of such an agent. No bacterium, rickettsia, fungus, protozoan or X-body was found which could be considered to be of aetiological significance. On the other hand, Swezy and Severin (45A) report the discovery of a rickettsia-like micro-organism in *Eutettix tenellus*, the vector of curly top of sugar beet. These workers state that they have found that *E. tenellus* harbours two different organisms, though these cannot be separated on morphological grounds. The difference between these two organisms is supposed to lie in the fact that one may be filtered through a fine Berkfeld filter, while the other is unable to pass through such a filter—the one being found in infective insects only and the other being present in both. There do not appear to have been any confirmatory observations since the foregoing statements were published. Paillot (25), in studies upon two species of aphis (*Aphis mali et al.*), has demonstrated in the blood and mycetocytes the presence of numerous slender rods which also occur in the eggs laid by the aphides. There is not, however, any evidence connecting these bodies with plant virus diseases. The writer has examined histologically the bodies of aphides infected with two potato viruses, paying particular attention to the salivary glands, and compared them with similar preparations of aphides known to be non-infective. He was unable to demonstrate any difference between the two. However, Blattny (5) considers that it is possible to detect a difference between the salivary glands of aphides, *Myzus persicæ*, which have fed upon leaf roll, mosaic and stipple-streak potato plants and of those of uninfective individuals. The difference is stated to lie in the areola around the cell nucleus of the salivary glands, which is dark in infective aphides instead of clear as in virus-free insects.

As regards insect vectors of animal viruses, there are one or two instances where a difference can be detected histologically between infected and virus-free insects. Hindle has related that the salivary glands of mosquitoes, *Stegomyia fasciata*, infected with the yellow fever virus are slightly larger than those of uninfected individuals. The presence of the virus of typhus in the louse, *Pediculus corporis*, can be detected by examining a film of the mid-gut and its contents for *Rickettsia prowazeki*. The connection between these rickettsiæ and the virus of typhus is best summed up in the words of Arkwright (46 p. 407) : " The virus and the rickettsiæ are so constantly associated that it is an almost inevitable assumption that the virus is identical with the rickettsiæ." In the case of Mexican typhus fever, the lice *Pediculus humanus* and *Polyplax spinulosus*, which transmit the virus, appear themselves to be actually diseased and usually succumb to infection by the rickettsiæ. On the other hand, certain species of fleas usually recover from the infection and appear able to regenerate the intestinal mucosa, partly destroyed by the rickettsiæ (23).

Non-inheritance of a Plant Virus by the Progeny of Infective Insect Vectors. The question whether a virus in an insect vector can be transmitted from parent to offspring is one of great interest and practical importance, and it has been investigated in several cases. First, as regards leaf hoppers, Severin (29) found that the vector of curly top (leaf) of the sugar beet, *Eutettix tenellus*, was non-virulent on hatching from the egg. Kunkel (18) states that the virus of aster yellows is not transmitted through the egg of *Cicadula sexnotata*, the specific vector of this virus. In streak disease of maize Storey (41) shows that the eggs laid by infective individuals of *Cicadulina mbila* produced uninfected progeny. Secondly, as regards aphis vectors, Elze (11) and the writer (36) have shown that the virus of potato leaf roll does not pass to the young of infective agamic females. Smith (34) and Bennett (3) state that no evidence has been obtained to indicate the presence of the raspberry-curl virus in the egg state of *Aphis rubiphila.* In the case of thrips vectors, it is considered very unlikely that the infective principle of tomato spotted wilt is transmitted through the egg stage of the two species of thrips, *Frankliniella insularis* and *Thrips tabaci* (27).

There is only one case on record where a plant virus is thought to be transmitted from parent to young, and this is described in the work of McClintock and Smith (21) on spinach blight and the aphides *Myzus persicœ* and *Macrosiphum gei*, where it was considered that the virus passed to the fourth generation. Hoggan, however (15), has recently repeated this work of McClintock and Smith, using the virus of cucumber mosaic (= that of spinach blight), and she finds no evidence of inheritance of the virus by the progeny of the aphis vectors. It is probable that the supposedly immune plants used by McClintock and Smith as hosts for the infective aphides were really susceptible to the virus, and in consequence each new generation of aphides picked up infection afresh. The non-inheritance of the infective principle of plant virus diseases by insects is paralleled by the infrequency with which plant viruses are transmitted through the seed of diseased plants (see p. 197).

On the question of the inheritance of animal viruses by the progeny of infective insect or allied vectors, the position is perhaps less decided. As regards yellow fever and *Stegomyia fasciata*, the question still remains unsettled. Hindle (46 p. 457) states that Marchoux and Simond (20) kept a female mosquito which laid its eggs sixteen days after an infective feed. These eggs were kept at a temperature of about 28° C. and were reared up to the adult stage. These insects were found to be infective and produced a typical attack of yellow fever when fed on a non-immune subject. No one has succeeded in repeating this experiment, and Bauer and Hudson (2), in Lagos, record feeding more than 200 mosquitoes, the offspring of infected parents, on a monkey with negative results. Hindle considers that it is possible that some special conditions are necessary for the virus to enter the eggs and be transmitted to the offspring.

As regards dengue fever, the virus is not transmitted to the offspring of an infected *Stegomyia* (32). Hereditary transmission of the virus of sandfly fever in *Phlebotomus* spp. is possible, since sandflies just emerged from the pupal stage may be infective (9). There are two alternative explanations, however, of such infectivity of the imagines: first, that the larvæ may have picked up the virus by feeding on the dead bodies of infected sandflies (49); and secondly that the virus may be conveyed among the sandflies themselves by the bites of certain mites (*Trombidium hindustaneium*) found on them (50).

More definite examples of virus transmission from adult to progeny are given by the cases of Rocky Mountain spotted fever and its arthropod vector, the wood tick *Dermacentor venustus*, and the Tsutsugamushi disease transmitted by mites. In the first case the virus is passed from larva to nymph, from nymph to adult and from the adult, through the eggs, to the next generation (46 p. 443), and in the second case the larval mite picks up the virus which is retained through the vegetable-feeding nymph and adult stages and passed on to the next generation of larvæ.

Extraction of a Virus from the Body of an Infective Insect Vector. By inoculation experiments with various organs of virus-infected insects it might be possible to throw some light on the question whether the virus is located in a particular organ, or whether it is spread uniformly throughout the insect's body. The writer (35) has performed experiments with this end in view, using hemipterous insects presumably infected with potato viruses. The salivary glands were extracted from aphides bearing the virus of leaf roll and mosaic respectively and inoculated into various healthy plants. These experiments gave negative results, as did also inoculation experiments with the body juices of large numbers of aphides (*Myzus persicæ*) infected with the Y potato virus. Possibly the juices liberated in macerating these aphides in a mortar inactivated the virus they contained. Smith (33) reports successful transmission of cowpea mosaic by needle inoculation with regurgitated sap and abdominal contents from infective beetles (*Ceratoma trifurcata*). McClintock and Smith (21) also report successful transmission of spinach blight by needle inoculation with the juices of infective aphides. In both these cases, however, the viruses are also sap-transmissible, and it has yet to be proved whether successful transmission can be accomplished by needle inoculation with the juices of infective insects in the case of a virus which is not normally transmissible by needle inoculation, such as potato leaf roll or aster yellows. Carter (7) has been able to transmit curly top of sugar beet by means of leaf hoppers which have fed on a suspension of crushed infective leaf hoppers in weak aqueous solution of various sugars.

Rambousek (26) has suggested that the cellular organ in aphididæ, known as the mycetome or pseudovitellus may play a part in virus transmission by this group. This organ, which has been studied by Uichanco (47), harbours symbiotic saccharomycetes. There does not, however, appear to be any evidence connecting the mycetome with plant virus transmission other than the fact that it occurs in the group Aphididæ which plays the largest part in the dissemination of plant viruses.

Hindle (46 p. 457) has shown that it is easy to infect monkeys with yellow fever by inoculation with the contents of mosquitoes which have ingested the virus. It is also possible to infect mosquitoes with the virus of yellow fever by feeding them on an emulsion of infected insects in syrup (20), a parallel experiment with that carried out by Carter with curly top of sugar beet and the leaf hopper *Eutettix tenellus*.

So far as the rickettsia diseases are concerned it is possible by staining to see this supposed organism within the body of the infected vector and to disseminate the virus, as in typhus, by means of the infective excreta of the insect.

Effect of the Sex or Degree of Maturity of an Insect Vector on its Ability to transmit a Virus. There seem to be few instances in the literature on differential transmission of plant viruses by the sexes in insects.

Storey (41) in studies of maize streak disease states that the infective power was unevenly divided between the sexes and was found more frequently in the females. There are, however, one or two interesting cases of difference in the ability to transmit between the larval and adult stages of the same insect vector. Kunkel (18), in studies on aster yellows, found that, although the young stages of *Cicadula sexnotata* were capable of picking up the virus, they were unable to transmit it because the so-called incubation period of the virus in the insect was longer than the nymphal life. If the nymphal period, but not the incubation period of the virus, was lengthened by exposure to low temperature, the nymphs were then able to transmit the virus. Kunkel also states that the incubation period of the virus within the insect is shorter in the adult than in the nymph. Certain thrips-transmitted viruses afford another case in point. Bald and Samuel, working in Australia with the disease known as tomato spotted wilt (1) and the thrips *Frankliniella insularis*, and Linford in Hawaii with *Thrips tabaci* and pineapple yellow spot (19), have shown that it is necessary for the thrips to feed in its larval form upon an infected plant in order to become a vector of the virus; the adult insect cannot pick up the virus *de novo*. The writer, working in England (39), has confirmed the above observation with tomato spotted wilt and *Thrips tabaci*.

It is somewhat difficult to understand the reason for this curious phenomenon, which is the exact opposite of that described by Kunkel on the insect relationships of aster yellows. Moreover, the virus of spotted wilt, unlike that of aster yellows, is sap-transmissible, which makes it unnecessary to assume a specific relationship between virus and vector. One cannot explain it, as in aster yellows, by assuming that it is due to the "incubation period" of the virus being longer than the—in this case—adult life of the vector because the maximum incubation period of the virus in the *larval* thrips is ten days, and the life of the adult is considerably longer than that. One can try to explain it on an anatomical basis, because there does appear to be a considerable difference in the width of the œsophagus, which is dilated in the larva but narrow and undilated in the adult. It might perhaps be suggested that owing to the narrow gullet of the adult the virus particles adsorb to the walls and so do not pass into the gut of the insect. It is at least interesting to remember that the virus of spotted wilt is very short-lived in extracted juice (four to six hours), and the œsophagus may be unsuitable for the virus to remain within it in a viable state. Incidentally, also, the spotted wilt virus is not filterable through candles which may indicate a high adsorptive capacity on the part of this virus. In considering these speculations it must be remembered that the adult thrips can transmit the virus of spotted wilt, provided it has been ingested during the larval instars of the insect. It is clear, therefore, that adsorption does not prevent the passage outward of the virus particles.

McClintock and Smith (21) find that spinach blight develops more rapidly in plants colonised with adult aphides (*Myzus persicœ*, *Macrosiphum gei*) than in those colonized with aphides in the first and second instars. These workers also obtained more infections with mature aphides than with the immature stages. The aphis *Myzus persicœ* seems equally capable of transmitting the virus of potato leaf roll as a larva as in the adult stage, provided that the necessary times for feeding and the waiting period of the virus in the body have elapsed.

Aphides in all stages of development are capable of acting as vectors of the viruses of red-raspberry mosaic and yellow mosaic of raspberries (4).

As regards differential transmission by the sexes in the case of vectors of animal viruses ; in those diseases where mosquitoes are the distributing agents, only the female is concerned because the male mosquito does not suck blood. Similarly with regard to differential virus transmission by the immature and adult stages of vectors, the mite *Trombicula akamushi* is able to disseminate the virus only in the larval stage, as the nymphs and adults are vegetable feeders and do not attack animals, but they pass on the virus to the next generation of larvæ (46 p. 439).

Relationship of Different Individuals, Strains and Varieties of Insects to Virus Transmission. The question of the relationship of strains, biological races and varieties of insect vectors, as opposed to different species, in regard to their ability to transmit viruses is a subject of great interest and one which has not been much investigated. It is not known whether all the strains of a particular species which transmits a particular virus can do so regardless of locality, country or previous host plant. The best example of variation within a species of ability to transmit a plant virus is given by some recent work by Storey on streak of maize and its specific insect vector, a leaf hopper, *Cicadulina mbila* Naude (43).

Races have been bred of this species which are, on the one hand, able, and, on the other, unable to transmit the virus of streak disease in the natural process of feeding on maize plants. Storey designates those insects which are able to transmit the virus as " active " races and those unable to transmit as " inactive " races. The cross, active female × inactive male, give an entirely active progeny in the F_1 generation. The F_2 generation of this cross consisted of active females and both active and inactive males. The reciprocal cross, inactive female × active male, resulted in the F_1 generation in only active females and inactive males. Both active and inactive males and females appeared in the F_2 generation. Storey offers the hypothesis that in the species *C. mbila* the male is heterozygous for sex, that the factor for activity is dominant to that for inactivity, and that this factor is linked with sex in the manner exemplified in the well-known instance of Drosophila. There seems little doubt that both active and inactive races judged by the usual criteria of the systematist fall into the one species *C. mbila*. There appear to be no morphological differences, internal or external, between the two races and, as shown above, crosses between them are fertile. Storey states that by certain treatments, he has been able to obtain infective hoppers from an inactive race, but this does not affect the general rule that in the natural process of feeding, inactive leaf hoppers do not transmit the streak virus.

Kunkel (18) found no cases of " inactive " leaf hoppers in the case of *Cicadula sexnotata* and the virus of aster yellows, but he did find that certain individual insects appeared to lose the infection after a short time, though the majority retained the virus throughout their life. Storey (41) reports a similar loss in infective power by certain individuals of the leaf hopper *C. mbila.* In the writer's studies upon the potato virus Y it has sometimes appeared as if the infective power of the aphis vector *Myzus persicæ* varied. So far as the virus of potato leaf roll and the aphis *M. persicæ* are concerned all strains of this insect seem equally capable of disseminating the virus.

As regards animal viruses, the view sometimes held that yellow fever is absent from the Orient owing to the local mosquitoes of the species *Aëdes ægypti* being biologically distinct and resistant to acquisition of infection is incorrect, as Hindle (13) has shown that an Indian strain of *A. ægypti* was able to transmit the infection in the same way as a West African strain of this species.

Relationship of Biting Insects with Plant Viruses. So far as the writer is aware, there is no record of a specific insect vector with biting mouthparts. There are three cases of plant virus transmission by biting insects (see p. 116), and in each case the virus is also sap-inoculable. It would seem, therefore, that biting insects play only a mechanical part in plant virus transmission, the infective principle being carried superficially on the mandibles or other parts of the mouth or retained temporarily in the alimentary tract. Elze (12), in attempting to classify plant viruses into groups based on their relationship with insect vectors, states that the potato flea-beetle, *Psylliodes affinis*, transmits the virus of potato leaf roll mechanically by the accidental adherence of virus to the mouthparts of the beetle. It is not made clear, however, how mechanical contamination of the mouthparts can be a mode of infection in the case of potato leaf roll where the virus is not mechanically sap-transmissible.

Attempted Production of Infection in an Insect Vector by Artificial Inoculation with a Plant Virus. In collaboration with Mr. J. P. Doncaster, the writer has endeavoured to inoculate the insect with a plant virus, and so to convert a non-infective insect into a virus-bearing one. These experiments have been performed upon the aphis *Myzus persicæ*, which is normally a vector of one or more viruses, and also upon other larger insects, such as capsid bugs, which do not normally transmit these agents. The viruses used were the potato viruses X and Y, and the insects were inoculated with a drop of virus sap or injected by means of a micro-pipette.

All these experiments have so far proved negative, chiefly perhaps owing to the high death-rate of the aphides treated, which in the majority of cases were unable to survive the injection process.

It was found, however, quite easy to scratch a drop of virus fluid under the skin of the aphis, provided the insects were kept in a saturated atmosphere. This method gave negative results, even with the Y virus, which the aphis normally transmits.

By developing a method for the inoculation by needle of fluids from and into insects Storey (44) has studied the manner of action of the maize streak virus in its vector, the leaf hopper *Cicadulina mbila.* Both the active and inactive races of this species—able and unable respectively to transmit the virus normally (see p. 154)—in feeding on a diseased plant ingest the virus, which may be recovered from the contents of the rectum. In an active insect, soon after its first feed on a diseased plant, the virus appears in the blood, and persists for a considerable period at least, even if the insect be removed from the diseased plant.

On the other hand, the virus remains confined to the intestine of an inactive insect and never normally passes out into the blood. Storey thus concludes that the appearance of virus in the blood is an essential step in the process of an insect becoming viruliferous, and that the reason for an inactive insect's inability to transmit lies in some property of the wall of the intestine that prevents the passage of the virus outwards into the blood. Since, however, the proportion of successes in the inoculation of inactive insects is significantly less than in similarly treated active insects, it is supposed that there is some secondary imperfect resistant mechanism the nature of which is unknown.

The resistance of the intestinal wall of inactive insects to the passage of the virus may be overcome mechanically by a puncture of the intestine, made by a needle introduced through the anus or penetrating the abdomen in the region of the stomach. Thus an inactive insect, punctured either before or immediately after a feed on a diseased plant, will often become viruliferous.

Storey advances experimental evidence for the belief that in such insects a part of the food ingested flows out through the hole in the intestine into the blood.

A considerable proportion of the *Cicadulinas* so treated survive the severely abnormal conditions produced.

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CHAPTER VII

INSECTS IN RELATION TO VIRUSES (*Continued*)

Some factors in the relationship between the plant and the insect vector.

Toxicity to the Plant of an Insect's Saliva in Relation to Virus Transmission

OWING to the method of feeding, which is uniform throughout the order, the saliva of hemipterous insects is injected into the plant during this process and so mixes with the plant sap. The saliva, so introduced, is thought to act as the vehicle of the virus (see Fig. 27). The toxic effect on the host of this injected saliva varies greatly in the different species of insect and is most pronounced in the Capsidæ.

As will be seen, however, it is not possible to correlate to any very great extent toxicity of saliva with inability to transmit a plant virus. The disturbances in the plant due to toxins or enzymes in salivary injections take a variety of forms and have probably been mistaken in some cases for virus diseases. The aphis *Myzus pseudosolani* when feeding upon tobacco produces yellow spots at the site of the puncture, and in addition causes a bunching and rosetting of the growing point, together with a twisting of the petiole (see Fig. 28). On potato the feeding of this insect does not give rise to spotting, but a marked rosetting of the growing points ensues (41). *Myzus circumflexus* and *Macrosiphum gei* also produce a darkening of the chlorophyll at the site of their feeding punctures in tobacco plants. *Myzus persicæ*, the proved insect vector of thirteen plant viruses, causes very little visible damage by its feeding, though it is possible to pick out by certain staining methods the track of its proboscis through the tissues (see Fig. 30). Davidson (10), in studying the effect of the feeding of *Aphis rumicis* upon plant tissue, found that the contents of the cells which were in contact with the saliva along the path of the stylets were extensively plasmolyzed and showed a movement of the disorganized cytoplasm towards the source of irritation. The contents of the cell become disorganized, the nucleus swells considerably, and eventually becomes a deeply staining, irregularly shaped, homogeneous mass. The cytoplasm becomes disintegrated, and in extreme cases only a small layer of cytoplasm is left

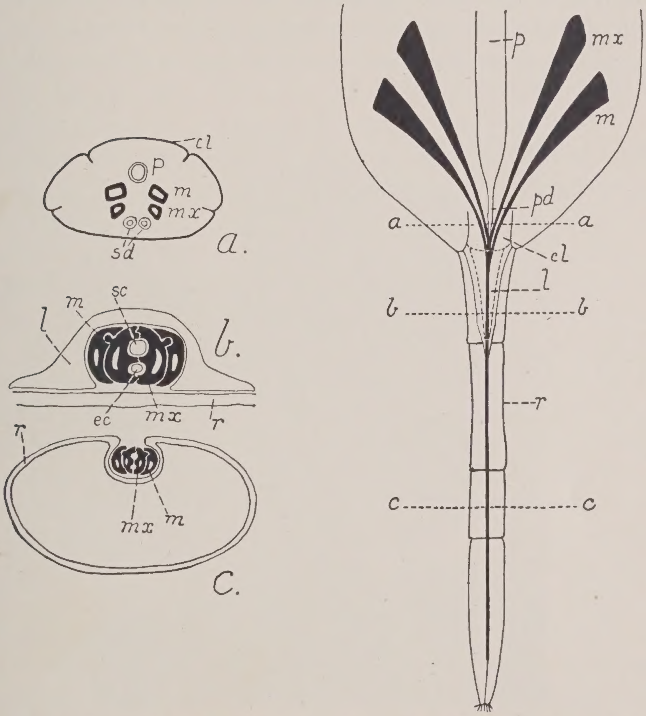

Fig. 27.—Diagram of the mouth-parts and adjacent region of the head of an hemipterous insect.

On the left are transverse sections across the regions bearing corresponding lettering; the magnifications of these sections are not uniform.

cl, clypeus; *ec*, ejection canal with salivary duct; *l*, labrum; *m*, mandible; *mx*, maxilla; *p*, pharynx; *pd*, pharyngeal duct; *r*, rostrum; *sd*, salivary ducts; *sc*, suction canal with pharyngeal duct. (After Imms.)

in the cell. The cells which are attacked eventually go brownish in colour, presumably owing to oxidase action. Even the lignified xylem vessels are acted upon by the saliva. The extent to which the local area attacked influences the surrounding tissues by a general distribution of the irritating salivary secretions is an important question, but there does not appear to be much spread of the damage to the surrounding tissue in cases of feeding by *Aphis rumicis* and *Myzus persicæ*. The saliva of *Aphis rumicis* is able to dissolve a passage through the walls of the cells for the piercing organ. It is also able to convert starch into sugar (10).

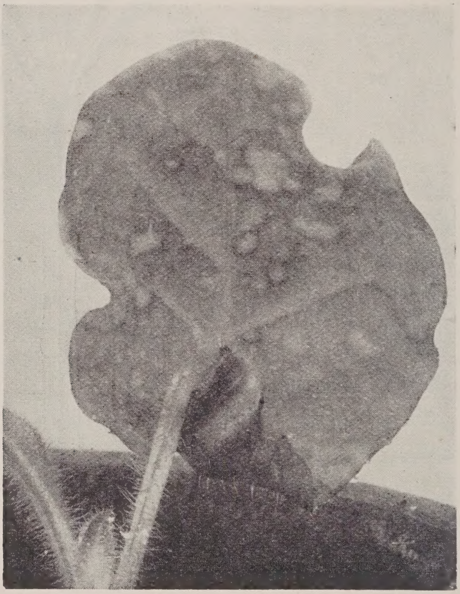

FIG. 28.—Tobacco seedling, var. White Burley, showing the toxic effect of the saliva of *Myzus pseudosolani* Theob. A chlorotic spot marks the site of each puncture and the petiole becomes twisted on itself.

As regards the effect of the feeding of various species of leaf hoppers (Jassidæ), the writer has studied the results of the salivary injections by *Eupteryx auratus* Liv. and *Erythroneura* (*Zygina*) *pallidifrons* Edw. Both these species produce white spots on the foliage of the plants wherever a puncture has been made. Sections through these spots show that the cell walls are intact, but the chlorophyll is decolorized and destroyed (see Fig. 33). It is suggested that this is due to the diffusion of a salivary toxin through the cell walls. Not all Jassidæ produce white spotting of the foliage when feeding, and this difference of effect may possibly be correlated with differences in feeding habit.

Floyd Smith and Poos (16) have studied the feeding habits of a number of species of leaf hopper of the genus *Empoasca.* They find that five of the six species studied produce definite spotting or stippling on the upper surface of the leaves, which is generally characteristic for each species. This stippling is considered to be due rather to a mechanical injury than to a toxin in the saliva. The effect of the feeding of the sixth species studied, *Empoasca fabæ* Harris, differs considerably from that of the others. This insect is known to cause " hopperburn " of potato and disease-like injuries on alfalfa (18) and many other plants. Smith and Poos state that when *E. fabæ* is confined on tender stems or petioles of apple, alfalfa or clover, wilting of the plant parts above the point of feeding occurs. When it is confined on the lower surface of the leaflet the mid vein and lateral veins become lighter in colour and wilting takes place in one or two days. Microscopical examination of sections through the leaves showed that the cells of the phloem are frequently punctured and are torn and distorted, and may be filled with the red-staining sheath material. The injury produced by *E. fabæ* is extremely localized, affecting only that part of the plant beyond the point of feeding, whether the injury is manifested as wilting or as yellowing or reddening. The explanation given by Smith and Poos for the injury produced by *E. fabæ* is as follows: It is suggested that the disorganization of the phloem elements may result in a disturbance of the plant processes above the point of attack, and that the production of substances by the plant itself may cause the partial or complete breakdown of the tissues involved. Smith and Poos think that a salivary toxin is not the most likely explanation of the injuries produced, but suggest that they are due to the interference with translocation of plant materials which produces either wilting when the xylem vessels are plugged in the process of the insect's feeding, or yellowing or reddening when the phloem is similarly disorganized and plugged. If these assumptions be correct, the differences in feeding effect between *E. fabæ* and the other five species of leaf hoppers studied are not due to chemical differences in the salivary secretions but to the fact that *E. fabæ* attacks the vascular bundles while the other five species feed on the mesophyll.

Johnson (22) has carried out a similar investigation upon the nature of the injury caused by *E. fabæ* to leguminous forage plants, and has come to much the same conclusion as that arrived at by Smith and Poos. Artificial inoculation with juice from yellowed and reddened leaves or from adults and nymphs of *E. fabæ* failed to produce symptoms of the injury, nor did symptoms develop on plants started as shoot-cuttings from yellowed and reddened plants. Confining an adult *E. fabæ* upon restricted areas of the leaf petioles of Ladino clover for eight, sixteen, and twenty-four hours resulted in the yellowing and reddening of about 20, 60, and 50 per cent. of the leaves respectively. With the twenty-four-hour feeding exposure an additional 40 per cent. wilted rapidly and turned brown. Symptoms almost identical with those caused by the feeding of the leaf hopper were produced artificially by mechanically severing all but one of the vascular bundles in Ladino clover petioles and by girdling Ladino petioles and alfalfa stems with steam. The chemical changes in leaves reddened and yellowed by artificial girdling and by leaf hoppers were essentially the same. These studies indicate that the reddening and yellowing caused by this leaf hopper are not the result of the injection by the insect of a specific inciting agent, virus, or toxin, but are caused by an over-accumulation of the carbohydrate products of photosynthesis, especially the osmotically active reducing sugars.

It is, however, in the Capsidæ that the saliva has its most toxic effect on the host. In the majority, though possibly not all, of plants fed on by such common bugs as *Lygus pabulinus*, *Lygus pratensis*, and *Calocoris norvegicus* the cells surrounding the puncture are killed outright and a large patch of dead necrotic tissue marks the site of each feeding puncture. These spots may enlarge somewhat after the Capsid has moved away, and occasionally the xylem vessels become clogged with a mass of darkly staining material (see Fig. 29). Painter (32) has studied the effects of punctures by a Capsid, *Psallus seriatus*, upon the cotton plant. He found that in the cells the cytoplasm showed a tendency to plasmolyze throughout the area on which the insects had fed. The chloroplasts and nucleus seemed to have been broken up and scattered through the cell, giving it a decided homogeneity. Cell walls in places both inside the leaf and on the outer epidermis had been ruptured, probably mechanically, without any dissolving action on the part of the saliva. The punctures of *P. seriatus* also produced swellings which occurred on the stems, petiole, midrib, and smaller leaf veins. In the centre of the swelling, apparently at the place where the insect feeds, there occurs a distinct destruction of some of the cell elements, while others enlarge to take their place. Painter describes certain intracellular inclusions (see p. 26), oval or round glistening bodies, which appear to move about in the cells of plants on which *P. seriatus* has recently fed. The nature of these bodies has not been determined.

A different type of damage to the plant host is associated with the feeding of Thrips species. This damage appears to the naked eye as silvery patches upon the leaf surface. According to Wardle and Simpson (44) the leaf injury consists essentially of necrosis of a patch of mesophyll cells lying immediately below a gash in an epidermal cell. Such necrosed patches are scattered and isolated when the infestation factor is low, but are confluent and form extensive rusty areas when the infestation is high. In later stages of leaf injury the necrosis involves the whole leaf, all the layers become disorganized, and premature shedding occurs. Wardle and Simpson consider that there is no evidence that the insects' salivary secretion is toxic to the plant.

In attempting to correlate the toxicity to the plant of an insect's saliva with capacity to transmit virus diseases, it is at least significant that the Capsidæ, one of the most numerous of plant-sucking insects, should be so little concerned with the dissemination of viruses. For a virus to multiply within a plant host it is essential that it should be introduced into the living cell, and this condition cannot be fulfilled by the feeding of many Capsid bugs owing to the lethal action on the plant cells of the injected saliva (see Fig. 29).

The mechanical difficulty of infecting a plant when the virus is accompanied by some chemical which is toxic to the plant is shown by inoculation of tobacco plants with virus extracts which have been preserved in 50 per cent. glycerine. The effect of the glycerine on the leaf tissue is very injurious, and this appears to prevent successful inoculation of the virus, a parallel case to transmission by Capsid bugs where the virus, if present, is accompanied by a toxic saliva. In the case of mechanical inoculation with virus preserved in glycerine, however, it is possible to ensure a high percentage of infection by immediately washing off the glycerine after inoculating the leaf (21).

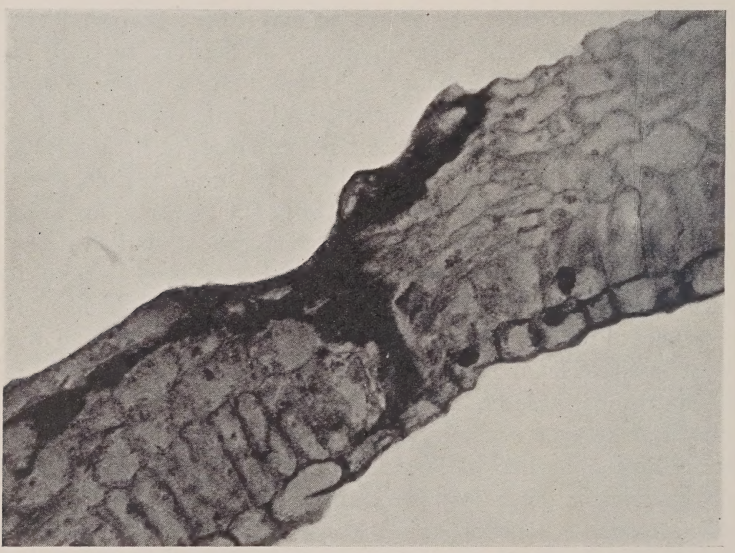

Fig. 29.—Photomicrograph of a section of potato leaf, fixed some hours after being punctured by a capsid bug, *Lygus pabulinus.* Note how the proboscis has penetrated the entire thickness of the leaf and the toxic saliva spreading to right and left. × 400.

The same phenomenon is shown by the difficulty with which infection is obtained with many sap-transmissible viruses if the leaf is lacerated or roughly excoriated at the time of inoculation. Sap-transmissible plant viruses as a whole are more easily transmitted by gentle rubbing than by the more drastic needle scratching. It is, however, essential that a wound be made, even if it is only the breaking of a trichome, in order to allow entrance of the virus.

As regard leaf hoppers, it is perhaps worthy of mention that those species which cause the local necrotic spotting when feeding appear to be seldom concerned with virus transmission while three important leaf hopper vectors of viruses, *e.g.*, *Eutettix tenellus* Baker, *Cicadula sexnotata* Fall., and *Cicadulina mbila* Naude, apparently do not cause necrotic spots (14). On the assumption of Smith and Poos, however, this difference of feeding effect is explainable rather by difference of *site* of puncture than by presence or absence of salivary toxins.

Carter (9) describes two types of leaf-spotting caused in pineapples by the feeding of the mealybug *Pseudococcus brevipes* Ckl. One is the usual chlorotic area which is characteristic of feeding by Coccids, the other type is designated " green-spotting." This latter type is caused by certain individuals only and is not common to the species. Attempts to transmit these green spots either mechanically or by transfer of non-green-spotting mealybugs to healthy leaves after feeding on existing green spots were unsuccessful. The ability to produce these green spots is transmitted to the offspring. The green-spotting mealybug can be differentiated from those unable to produce green spots by a difference in body colour, a dark-brown colour of the body fluids that imparts a greyish cast to the waxy covering of the insect. Carter considers that the hereditary nature of the ability to produce green spots leads to a consideration of the possible relationship between the intracellular symbionts of the insect and its secretions.

To conclude, then, it is probably true to say that the degree of toxicity to the plant of an insect's saliva can be correlated in a few cases only with ability or inability to transmit plant viruses.

In all the foregoing cases the damage to the plant host caused by salivary toxins has been a purely local condition. There are, however, one or two interesting cases of systemic infection of a plant apparently caused by the injected salivary toxins of insects. The most thoroughly investigated case of this kind is known as " pineapple wilt " and has been described by Carter (8).

There appear to be two forms of pineapple wilt, and their relationship with the pineapple mealybug, *Pseudococcus brevipes*, has been conclusively demonstrated. The wilt disease is a form of collapse, its precise form depending upon various factors, such as the size and time of onset of the initial mealybug population and the age of the plant attacked. Recovery in various degrees is commonly encountered. " Quick wilt " develops after a sudden attack by a fairly large colony of mealybugs, which may, however, feed for a short time only. " Slow wilt " is the result of a continuous attack by a gradually increasing population of mealybugs. Its incidence is governed by the size of the mealybug colony and the length of time the colony feeds on the plant.

The disease is believed to be due to the secretion by the insect of a non-living but toxic principle that is variably diffusible, sometimes affecting only the area of leaf actually fed upon by the insects, sometimes producing wilt in two or three contiguous leaves, or, as is usual under field conditions, a wilted condition of the entire plant. Comparison of quick and slow wilt suggests an antitoxic reaction by the plant which enables it to accommodate itself to large but gradually developed populations of the mealybug. The toxicity of the saliva of *Pseudococcus brevipes* appears to vary with the kind and condition of the insect's host plant. This is believed to be due to changes in the insect's secretions induced by the varied nutritional conditions afforded by the host plants. The chief difference between pineapple wilt and a true virus disease lies in the fact that the toxin producing pineapple wilt is incapable of multiplying in the plant.

An apparently similar case of systemic infection with a salivary secretion is known as " yellows " of beans (42), and is associated with a new species of leaf hopper (*Empoasca fabalis* n.sp. Delong.). The symptoms vary markedly in different varieties and species of plants, but the chief manifestation is a yellowing of the foliage.

In some cases the leaves become almost white and the plant assumes a generally anæmic condition. There is not very much exact information on the nature of " yellows " of beans, but it does not appear to be a true virus disease and is closely associated with the feeding of *Empoasca fabalis*.

Richards (33) describes a disease of potatoes in Utah, also known as " yellows," which is connected in some way with the potato psylla, *Paratrioza cockerelli*. This condition is apparently systemic; at any rate it is propagated by the tuber in some cases. It does not, however, seem clear whether potato yellows is a virus disease or a systemic infection with a toxin.

A further investigation of the above " psyllid yellows " of the potato has recently been carried out (34). The symptoms of this disease under conditions of unmodified sunlight consist of yellowing, basal leaf rolling, and purpling of the younger leaves, yellowing and rolling of older leaves, nodal enlargement, increased axillary angle, aerial tubers and shoots, frequent rosetting, and various apical growths and distortions. The disease is not mechanically transmissible, nor, according to this later work, is it tuber-propagated. This disease, while appearing similar to that caused by *Empoasca fabæ*, differs from it in that the adult form of *Paratrioza cockerelli* is not capable of producing the symptoms which arise only after feeding by the nymphs. The nymphs, however, are capable of producing the disease immediately after hatching. " Psyllid yellows " therefore may be an example of a disease induced by a toxin which is produced or injected by the psyllid nymph during the process of feeding.

A serious disease of sugar cane is associated with a Cercopid, *Monecphora* (*Tomaspis*) *saccharina* Dist., and is known as " froghopper blight." The saliva of this insect is slightly acid (*p*H 6·0–6·2), it has a distinctively diastatic action, converting starch into soluble sugars, and it contains oxidases. Withycombe (48) has studied in detail the effect of the froghopper's sucking upon the tissues of the sugar cane. There occurs a distinct bleaching of the chlorophyll at the side of the puncture, but the most noticeable effect is upon the contents of the border parenchyma cells. According to Withycombe, the spread of injury from a froghopper puncture can be explained somewhat as follows: First, besides the direct injury and drain upon the contents of the vascular tissues there is a marked injurious effect upon the border parenchyma, with removal of carbohydrates, etc., stored temporarily therein. The injury to border parenchyma is extensive longitudinally; the immediate effect upon chlorenchyma is more localized. The injury produced by the saliva is effected by several ferments, among which diastatic and oxidizing enzymes have been demonstrated, and there are probably others. The metabolic equilibrium of the plant is upset and the rate of respiration probably increases locally with oxidation. Super-normal oxidation with the formation of red pigment occurs from the action of plant oxidases possibly aided by the oxidase in the insect's saliva. Loss of sap with its contained food materials from the vascular tissues can be made good by translocation from elsewhere, but local translocation has been interfered with and probably arrested by the injury to the border parenchyma sheath. This results in reduction of water supply, with solutes, to the chlorenchyma and other neighbouring tissues, and unless loss of water, etc., is reduced by decreased transpiration a local shortage in the tissues is likely to result. There is, however, no decrease in the transpiration rate. Interference with translocation is further intensified when the phloem becomes blocked. Water deficiency prevents recovery of the protoplasts in all affected cells and culminates in a drying up of the tissues. Assuming continued effect of the enzymes introduced by the froghopper, the metabolic equilibrium of the plant will continue to be upset unless a sufficiently powerful counter-reaction is forthcoming. This counter-reaction is likely to be impeded by difficulties of conduction. Withycombe conducted experiments in order to ascertain whether the froghopper was a vector of any infective principle which might account for the sugar cane blight. He was unable to spread the disease by mechanical means, and, what is more important, he showed that froghoppers, bred from the egg on healthy plants, were still, as adults, capable of producing the typical leaf injury. There seems little doubt that " froghopper blight " of sugar cane is primarily due to the toxic effect of the insect's feeding.

So far as the writer is aware none of the insects described in this section as producing what may be called " toxin diseases " of plants have been incriminated as vectors of plant viruses.

There appear, therefore, to be two types of disease in plants other than virus diseases which follow directly upon the feeding of insects. One is the diffusible toxin disease exemplified in pineapple wilt, and possibly psyllid yellows of potatoes, due to injected salivary secretions, and the other is " hopperburn " of potatoes and perhaps " froghopper blight " of sugar cane where the disease is apparently due to interference with translocation of plant materials, which produces either wilting, when the xylem vessels are plugged in process of the insects' feeding, or yellowing and reddening when the phloem is similarly disorganized.

Methods of Feeding of Insects in Relation to Virus Transmission

Since infection of a plant with a virus depends to some extent on the manner of application of the fluid containing the virus and perhaps also on the exact site of its introduction, it will be of interest to consider the methods by which the various hemipterous insects feed and to try to correlate to some extent feeding methods with ability to transmit a virus. It is proposed to discuss this problem and other subsidiary points under the following heads :—

(1) Methods of penetration of the plant tissue by the insect and particular region tapped.

(2) Can a virus be picked up and transmitted by an insect feeding on any part of a plant ?

(3) Ability of an infective vector to feed upon a susceptible plant without transmitting the virus.

(4) The length of time necessary for an insect to feed upon its host to pick up and to transmit a virus.

The type of sucking mouthparts possessed by the Hemiptera as a group is shown and explained in Fig. 27.

Methods of Penetration of the Plant Tissue by the Insect and Particular Region Tapped. Various studies have been made upon the methods of feeding of hemipterous insects and the particular region of plant tissue sought by them. Busgen (4) finds, for aphides, three types of stylet track, namely :—

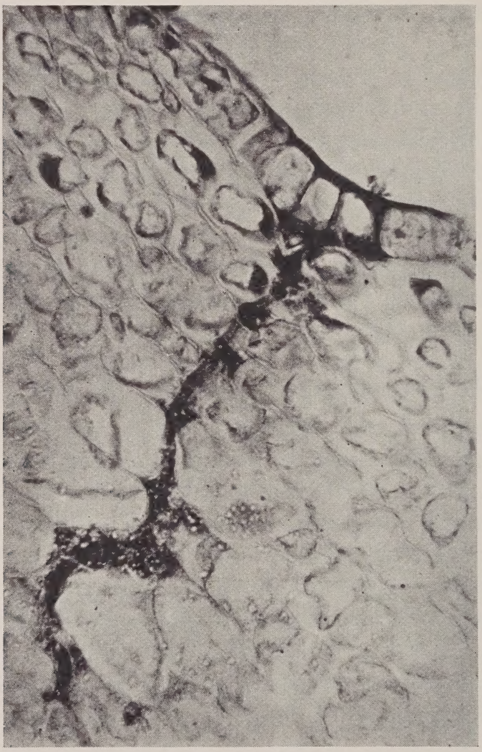

Fig. 30.—Photomicrograph of the stylet track of *Myzus persicæ* Sulz. in the tissue of a potato stem. Note the intercellular course of penetration. The disintegration of the cell contents near the stylet track may be seen. × 800.

(1) Intercellularly to the phoem ;
(2) Intracellularly through the parenchyma (no phloem objective) ;
(3) Intracellularly to the phloem.

The writer (36) has made an intensive study of the feeding methods of a representative group of hemipterous insects with a view to finding a possible correlation between feeding and virus transmission. Three species of aphides were studied: *Myzus persicæ*, *Myzus circumflexus*, and *Macrosiphum gei* (= *solanifolii*).

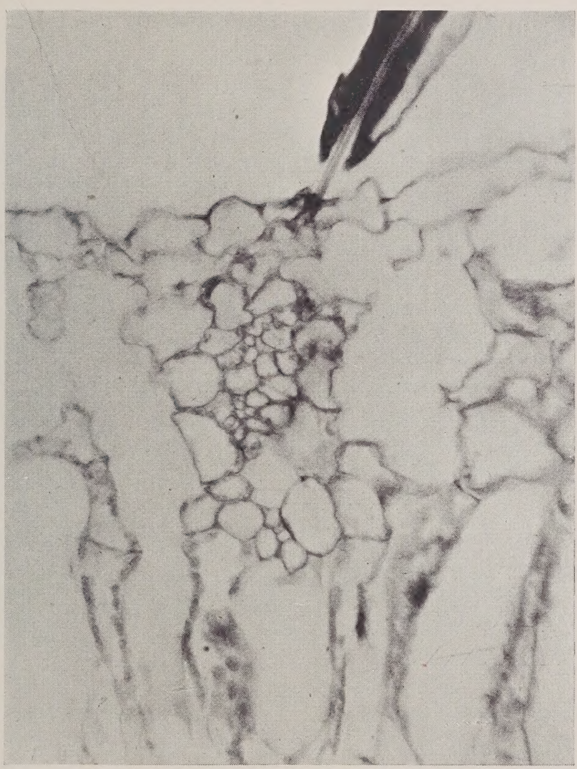

Fig. 31.—Photomicrograph of a section of potato leaf, showing the stylets of the aphis, *Myzus persicæ* Sulz., in the act of penetrating a stoma. The objective is the phlœm which can be seen just below. × 800.

The stylet tracks of these aphides were intercellular and directed to the phloem (see Figs. 30, 31), though occasionally the stylet track of *M. gei*, which is a large aphis, was intracellular. The track of the whitefly *Asterochiton vaporariorum* (Aleyrodidæ) was also made intercellularly to the phloem. The stylets of a scale insect, *Aspidiotus*, passed intracellularly through the parenchyma without having the phloem as a necessary objective (see Fig. 32), while the mealybug *Dactylopius* penetrated intracellularly to the phloem. The leaf hoppers studied fed mainly in the mesophyll, though both these and the Capsid bugs appeared also to tap the vascular bundles (see Fig. 33). In a later study the writer (41) has examined the stylet tracks of another aphis, *Aphis rhamni*, which is also a phloem feeder.

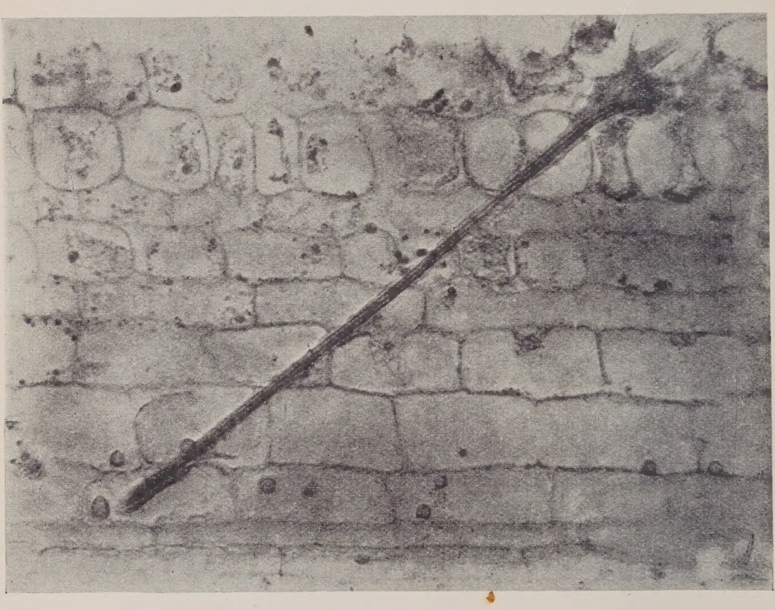

Fig. 32.—Section of leaf of *Cordyline* showing the stylets of *Aspidiotus* sp. (Coccidæ) *in situ*. The intracellular path followed is well shown. Note the saliva enveloping the stylets on each side and the stylet track, exposed where the piercing organ has turned aside. × 800.

Davidson (10), in studies on the feeding methods of *Aphis rumicis*, shows that the piercing organ passes intercellularly through the cortex, only occasionally passing through individual cells, until eventually it reaches the vascular bundles. The phloem elements of the vascular bundles are the chief source of the food supply, although other cells of the plant may also be tapped for nourishment.

All aphides do not necessarily tap the phloem for their nourishment. Petri has shown that *Phylloxera* on the roots of the vine taps the cortical cells and does not reach the vascular bundles.

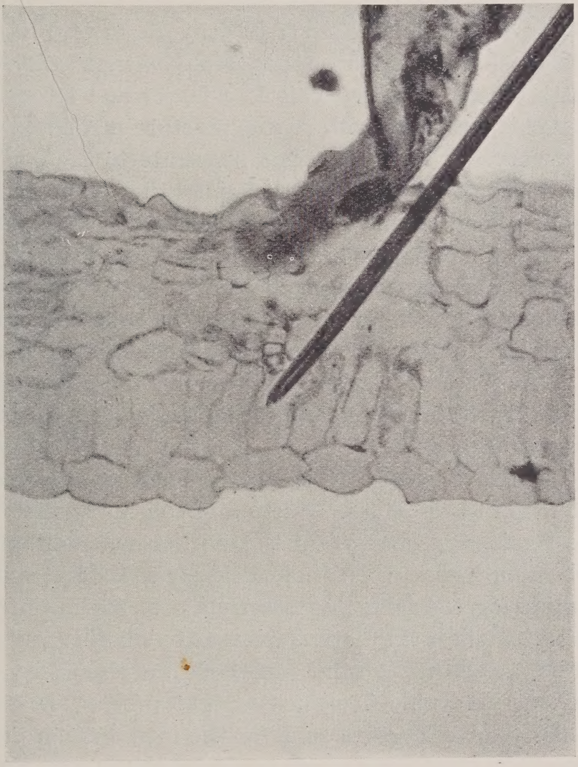

Fig. 33.—Stylets of a leafhopper tapping the palisade cells of the potato leaf. Note the destruction of the chlorophyll to the left of the stylets. The path of penetration is intracellular. × 600.

Floyd Smith and Poos (16) have studied the feeding habits of six species of leaf hoppers of the genus *Empoasca*. They find that while five of these species are primarily mesophyll feeders, the sixth species, *Empoasca fabæ*, feeds only on the phloem or water-conducting tissues. Floyd Smith and Poos consider that it is this difference in feeding-method which is responsible for the severe injury produced in the host by *E. fabæ* and not the presence of toxins in the saliva. The stylet track of this leaf hopper appears to follow an intercellular course. Withycombe (48) has studied the feeding habits of a froghopper, *Monecphora saccharina* Dist., which attacks sugar cane. This is a larger and stronger insect than the aphis, and the depth of the puncture made and the path it follows vary. Often the stylets penetrate to the epidermis of the opposite side of the leaf, at other times they go no deeper than the neighbourhood of the vascular bundles. There appears to be no preference for upper or lower surfaces of the leaf.

A different mode of feeding is illustrated by the methods of the thrips, which gashes an epidermal cell by pickaxe-like movements of the single mandible, induced by a slight rocking movement of the head. In most cases the mandible can only affect the outer epidermal wall, the inner wall and the lateral walls of the mesophyll cells being broken down by similar movements of the maxillary laciniæ, which are protruded further. There is no evidence of attack through the stomata. Suction of the chloroplasts into the pharynx is aided probably by the partial vacuum established within the mouth cone when applied closely to the leaf surface (44)

In considering the foregoing description of feeding methods of the various insects, the question arises as to whether any relationship can be traced between those methods and a capacity for virus transmission. The phloem elements might, perhaps, be considered a particularly advantageous spot for the introduction of a virus, as they contain substances which might be suitable for the growth of a hypothetical organism and would also facilitate dispersal about the plant. All the aphides studied were phloem feeders and the aphides are the most efficient insect vectors of plant viruses. Nevertheless the fact of phloem feeding does not seem important for the following reasons : the virus disease known as leaf roll of potato is primarily a phloem disease, and the three aphides *Myzus persicæ*, *Macrosiphum gei*, and *Aphis rhamni* are all phloem feeders. In spite of this, however, the first-named only is an efficient vector of the virus and appears to have a special affinity for it, an affinity which must be based on some factor other than method of feeding.

As regards leaf hoppers, none of those species, of which the feeding methods have been studied, appear to be vectors of plant viruses, and it is therefore difficult to draw any conclusions from a consideration of their feeding habits. There is no reason, however, to suppose that known leaf-hopper vectors, such as *Cicadula sexnotata*, *Cicadulina mbila*, or *Eutettix tenellus*, feed in a manner markedly different from that employed by *Empoasca* spp. or *Eupteryx auratus*. There may, of course, exist the same difference in *locality* of feeding as Floyd Smith and Poos have shown to exist in *Empoasca* spp., but this question must await further investigation. The whitefly *Asterochiton vaporariorum* has not yet been shown to be a vector of virus diseases, but at least one, and possibly two, species of Aleyrodidæ do transmit viruses, and it is to be presumed that these latter insects have a similar intercellular stylet track leading to the phloem as is possessed by *Asterochiton vaporariorum*.

The scale insect which was shown to have an intracellular stylet track through the parenchyma without a phloem objective is not a vector of viruses, nor is *Dactylopius*, the mealybug which does tap the phloem. It may be worth recalling that it is a mealybug which is the cause of the systemic toxin disease of pineapples described by Carter (8). So far as the writer is aware, there is no well-documented case of virus transmission by any of the Coccidæ.

The Cercopidæ (froghoppers), as exemplified by *Monecphora saccharina*, which sometimes tap the vascular bundles and sometimes penetrate to the opposite epidermis, are also not concerned with plant viruses. The Capsidæ (Miridæ), which may be said to feed in a similar manner to the Cercopidæ, have been incriminated in one or two cases as vectors. There remain the thrips, which, with their characteristic scraping of the epidermis, are unable to reach the vascular bundles.

From the foregoing facts, then, the conclusion must be drawn that there is no correlation between method of feeding and ability to transmit a virus, except possibly in the case of the thrips.

These insects transmit the virus disease of tomatoes known as spotted wilt. This virus is also sap-transmissible and infection with it is best obtained by a light rubbing movement rather than an injection—conditions which the thrips, by their method of feeding, fulfil more closely than any of the Hemiptera. In theory the only insect which would be precluded by its method of feeding from disseminating a plant virus would be one which tapped only the xylem vessels, since it has been shown that the virus, once injected into the water-conducting elements, is unable to escape therefrom except through a rupture in the walls (5).

Can a Virus be Picked Up and Transmitted by an Insect Feeding on Any Part of a Plant? The answer to the first part of this question is obviously closely connected with the systemic nature of plant virus infection, and there is little doubt that viruses, in some cases at all events, are not uniformly distributed throughout the plant. That variation in virus concentration in the host occurs has been shown in several cases. Storey (43), working with streak of maize, found that the virus was usually obtained by colonizing the insect vector upon the chlorotic areas, but not, as a rule, after colonization upon the green areas. Henderson Smith (19) reports the apparent localization of a virus in *Datura stramonium* to certain small areas from which alone infection could be obtained. McKinney (30) also is of the opinion that virus concentration varies in different parts of the same plant, in plants grown under differing conditions, and in plants of different ages. The writer, working with the X potato virus and that of tomato spotted wilt, has on several occasions observed marked localization of symptoms to one leaf of the host plant. This is especially true of the spotted wilt virus on *Solanum capsicastrum*, and inoculation from other parts of the plant on which no symptoms appear has failed to give positive results. It has frequently happened with this virus that inoculation to tobacco has produced a local infection of the inoculated leaf without further spread to the rest of the plant.

The virus of bunchy top of tomato is generally distributed through the stems, leaves, and roots of a diseased plant, but is absent from the old, apparently normal, leaves (28). As a general rule, however, it may be postulated that the virus can be ingested by an insect feeding on any part of a plant other than the seed, and in some cases the roots. There are exceptions to this rule, exemplified by streak of maize. As regards the second part of the question, there seems little doubt that infection of a healthy susceptible plant will ensue if an infective insect feeds on any part of the plant—again, with the possible exception of seeds and roots.

With regard to infection by the roots, Mulvania (31) attempted to transmit the virus of tobacco mosaic by the following three methods: placing uninjured roots in contact with the juice of plants affected with the disease; inoculating the virus by means of the needle into the roots, and by applying infective juice directly to the surface of freshly cut roots. No infection took place in 150 plants by any of these three methods. On the other hand, the writer (39) has infected tobacco plants with a potato virus by needle inoculation into the roots. When uninjured roots were placed in the infective juice, however, no infection took place. The writer has also shown (38) that the virus of potato leaf roll can be equally well disseminated by the aphis vector feeding upon the sprouted tuber, on the leaves and shoots, and on the stem alone.

Ability of an Infective Vector to Feed Upon a Susceptible Plant Without Transmitting the Virus. There are several recorded instances of the ability of an infected insect to feed upon a plant host without transmitting the virus. The writer (41) has found that aphides, known to be bearing the virus of potato leaf roll, when fed upon a progressive series of healthy plants, occasionally failed to infect plants in the series. Samuel *et al.* find that infective thrips do not always infect with spotted wilt every tomato plant on which they feed (35), while Carsner and Stahl (7) report a similar occurrence in studies on curly top of the sugar beet. It must be understood that the above cases do not refer to the period of non-infectiveness which occurs in the vector during the " incubation period " of the virus in the insect (see p. 134).

As regards animal viruses, Hindle (20) states that mosquitoes which have ingested the blood of yellow fever patients do not invariably become capable of producing infection by their bite, even when the virus can be shown to be present in their bodies. Experiments have shown that the virus of yellow fever may persist in mosquitoes, kept at low temperatures immediately after feeding, without their bite becoming infective. Subsequent exposure to a temperature of 28° C. causes the bite of such insects to become infective.

The reason for the occasional failures in transmission of the plant viruses may perhaps lie either in unusual reactions on the part of individual host plants or in a temporary interruption in the flow of the virus from the insect.

The Length of Time Necessary for an Insect to Feed Upon its Host to Pick Up and to Transmit a Virus. These times have been investigated in a few cases. Storey (43) shows that leaf hoppers can acquire the maize streak virus after one hour's feeding. The writer (41) finds that *Myzus persicæ* can acquire the virus of potato leaf roll after six hours' feeding on a diseased plant, and that a known infective aphis can infect a healthy potato plant after two hours' feeding. Samuel *et al.* (35), working with spotted wilt of tomato, state that infective thrips may inoculate a plant after six hours' feeding, shorter periods not having been tested.

Uninfected adult whiteflies (*Bemisia gossypiperda*) can pick up the virus of leaf curl of cotton from crinkly Sakel cotton in just over three hours, and possibly in a shorter time. Transmission was, however, only obtained on two plants out of five, when the time available for picking up the disease was three hours and twenty minutes. When it was four hours and thirty minutes or longer, transmission was obtained in every case. Infected whiteflies can transmit the disease to healthy Sakel plants in thirty minutes and the whole process of infection of the whiteflies and of the healthy plant has been accomplished in six hours and thirty minutes (25).

The aphis *Amphorophora rubi* can acquire the virus of yellow mosaic of raspberries in a feeding-time of two hours and transmit it to healthy plants during the following forty-eight-hour period. It can acquire the virus of red-raspberry mosaic in twelve hours and transmit it to healthy plants during the following twelve-hour period (3).

The shortest period of feeding required by an infective insect to transmit a virus is described in the work of McClintock and

Smith (29) on spinach blight, where virus-bearing aphides produced infection in healthy plants when allowed to feed upon them for two minutes. The same workers state that non-infective aphides must remain on diseased plants from five minutes to fourteen hours in order to become vectors of the virus.

Doolittle and Walker (12) state that the melon aphis, *Aphis gossypii*, can pick up the virus of cucumber mosaic after feeding for five minutes on a diseased plant and transmit it after feeding on a healthy plant for a similar period.

Relationship of the Numbers of Infective Insects to Subsequent Virus Infection of the Host

The relationship of the numbers of infective insects used, to the resulting disease in the plant can also be regarded as the effect upon the plant host of varying the dosage of virus, *i.e.*, from the minimum dose delivered by one insect up to a massive dose delivered by a large number of insects. In other words, does the disease produced by the virus injected by many infective insects differ in any respect from the disease produced by the same virus injected by a single insect ? Storey (43), working with streak of maize, finds that in comparative series of infection experiments with groups of leaf hoppers and single individuals, the average incubation period was shorter in the group series, but that there was no difference in the minimum incubation period in each series. The severity of the disease produced in each case was the same. Storey considers that the effect of a group of leaf hoppers is the effect of the most " powerful " individual of the group, and is not the average effect nor the sum of the effect of the members of the group.

The writer (38), working with potato leaf roll, finds that in comparative infection experiments with numbers of aphides varying from two to eighteen, one or two virus-bearing aphides were capable of infecting a plant with leaf roll. Such infection, when achieved, differed in no degree of severity from that induced by eighteen aphides. The incidence of infection was greatest amongst plants colonised with the larger numbers of aphides.

2-18

Bennett (2) was able to transmit raspberry leaf curl by means of a single individual of the aphis, *Aphis rubiphila.* He also found the percentage of infection higher when numbers of insects were employed.

Carsner and Stahl (7), in regard to curly top of the sugar beet, state that ten or more insects are more likely to produce the disease in an inoculated plant than when inoculation is made with one or two insects. In cases where the disease is produced, there is no difference in the length of the incubation period or the severity of the effect between inoculations with single insects and those with ten. Fajardo (15) comes to similar conclusions with bean mosaic. All these experiments tend to show that there is a minimum dosage of virus necessary to produce infection, and any excess of that amount has no effect upon the severity of the resulting disease.

It is possible, however, that in certain cases where a large quantity of inoculum is used there may be an increased local reaction, and this is more likely to take place when the inoculation is made mechanically. Dickson (11) says in this connection : "The amount of inoculum, and its virulence, affects the symptoms inasmuch as leaves developing immediately after heavy infection of seedling plants are deformed and savoyed, while frequently those growing later show ordinary, or perhaps only slight, infection."

Carsner and Lackey (6) consider that the result of an inoculation, that is to say, infection or non-infection, and usually the length of the incubation period, depends on the proportions of the chemical substances actively entering into the reaction.

In some experiments with the attenuation and restoration to virulence of the virus of sugar beet curly top, it has been demonstrated that the greater the number of insects used to inoculate the beet the greater is the percentage of infection obtained. It is also apparent that two leaf hoppers carrying virulent virus may cause a higher percentage of infection and severer symptoms than ten insects carrying attenuated virus. Since as great a number as eighty leaf hoppers with the attenuated virus fail to produce severe symptoms on young growing beets, while one leaf hopper carrying virulent virus will produce severe symptoms, it is concluded that in experiments of this type the dosage of virus is not a factor controlling the subsequent symptoms produced (27).

On the other hand, Wille (47), working with leaf crinkle of sugar beet and the plant bug *Zosmenus quadrata* Fieb., states that the disease is influenced by the number of bugs on a plant, the duration of their feeding, and the age of the plant. Severe crinkle can be produced by ten bugs feeding for two hours or by three bugs for twenty-four hours. The same writer also finds that the disease develops most rapidly if the bugs are numerous, their feeding prolonged, and the temperature high. Wille evidently considers that the number of insects, and consequently the amount of virus dosage, does affect the intensity of the resulting disease, and that numbers are not, as in the foregoing cases, merely instrumental in making development of the disease more certain.

Dissemination of Viruses from Plant ''Carriers'' by Insect Vectors

One of the most interesting phenomena associated with plant viruses is the existence of " carriers," *i.e.*, plants which, though symptomless and apparently healthy, yet harbour one or more concealed viruses within them. It is quite easy to transmit a concealed virus from such a plant to one more susceptible by artificial means such as needle inoculation or grafting, according to the nature of the virus. The question, however, as to how far insects are able to disseminate viruses to susceptible plants from such carriers is one of great interest and practical importance, and has been very little studied. The two plants in which the phenomenon is most commonly found are the potato and the hop, and the existence of such latent viruses adds greatly to the difficulty of the successful study of viruses in relation to those plants. Needle inoculation from carrier potato plants into healthy tobacco produces a necrotic disease (J. Johnson (23) and K. M. Smith (37)), and the writer has found that the aphis *Myzus persicæ* will transmit a virus from a symptomless carrier potato to tobacco. It has also been shown (40) that the same aphis will transmit a virus (= Y) from the Solanaceous weed, *Solanum nigrum*, which is capable of acting as a carrier, to healthy potatoes (see Fig. 34).

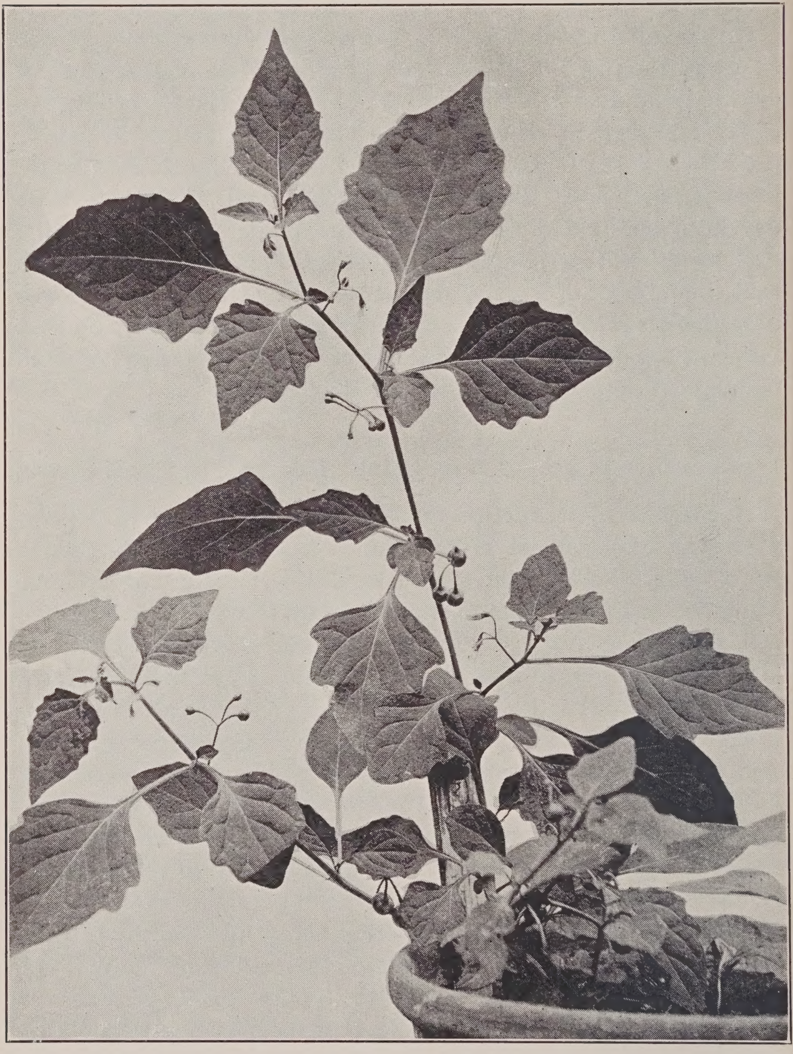

Fig. 34.—*Solanum nigrum*, the black nightshade, carrying, without symptoms, the potato virus Y (see Fig. 50).

The work of Salmon and Ware (34A) and Thrupp (43A) has proved that grafting of carrier hops on to healthy susceptible varieties will transmit the mosaic virus, which in the case of the hop is a virulent and often fatal disease. The insect vectors of hop viruses have not, however, been studied in this country, save for some preliminary work by the writer, so that it is not possible to say what part is played in nature by insects in the dissemination of viruses from carrier hops. Another example of a plant virus carrier is *Chenopodium murale*, which under certain circumstances harbours the sugar beet virus, curly top. Certain individual plants of sugar beet also appear capable of harbouring the virus without symptoms (26). There is no information on the question of transmission by insects from these latter plants.

Transmission of Plant Viruses by Soil-inhabiting Insects.

There are very few authentic records of virus transmission by soil-inhabiting insects. The question has been raised on several occasions with regard to potato leaf roll, and Whitehead (46) considers that he has some positive evidence, but the only definite statement appears to be that of Elze (13), who claims to have transmitted this virus by means of the Dipterous larvæ, *Tipula paludosa*, working below soil level, and this requires confirmation (see also Folsom, Schultz, and Bonde (17), and Atanasoff (1)).

Johnson and Ogden (24) have shown that the virus of tobacco mosaic may persist in the soil for long periods and McKinney (30) finds soil-transmission of tobacco mosaic to take place in the absence of all soil fauna. How far, if at all, soil insects are concerned in transmitting this virus is not known, and further research is required on this and on the question of insect vectors generally of tobacco mosaic. Webb (45), working on the soil relationships of mosaic disease of wheat, finds that infection of seedlings with this virus may and does occur through either the roots or the crown of the plant, or both. He also states (45A) that it is possible that some soil vector of an animal nature is active in the promotion of the disease, but that so far there is no evidence of such a vector.

On the whole it is probably true that soil-inhabiting insects, and soil transmission generally, play a very small part in the spread of plant virus diseases.

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CHAPTER VIII

TRANSMISSION AND SPREAD OF PLANT VIRUSES

Transmission of plant viruses by agencies other than insect vectors : by grafting, by inoculation, by seed, by pollen, and by contact of foliage or roots—Factors governing the spread of certain plant viruses in the field.

Transmission by Grafting

THE first method of virus transmission to be discussed is that of grafting, where actual organic union is effected between scion and stock. All plant viruses are transmissible by grafting, and there are certain viruses causing infectious chloroses which appear to be only transmissible by this means, although it is possible that there exist insect vectors or other modes of transmission in nature which have not yet been discovered.

It is proposed to discuss shortly various methods of grafting as they have been used in the experimental transmission of viruses affecting different types of plants. Succulent herbaceous plants such as potato, tomato, and tobacco plants may conveniently be cleft-grafted as follows : take a young shoot for the scion and cut off the larger leaves ; then pare the stem on each side with a sharp scalpel until it assumes the shape of a wedge. Next cut off the top and slit the stem of the stock, taking care that it is young and sappy and not hollow ; insert the scion and bind lightly with thin rubber tape, such as is used for making the interior of golf balls. This rubber tape was first used by Salmon and Ware for grafting the hop plant (62). Finish off the binding with a few drops of the ordinary rubber solution used for bicycle tyres. Then place the plant in a humid atmosphere for about ten days. It will be found that the rubber tape gradually perishes and falls away by the time organic union is effected, so that there is less fear of constricting than if raffia were used.

Young and Morris (79) have used three methods of stem grafting for the transmission of witch's broom of potatoes and tomatoes: first, cleft grafts as described above; secondly, the insertion of slips into slits made in the stem of the stock; and, thirdly, inarch grafts which were made by slicing off the cortical layers on one side of each of two stems and binding the cut surfaces together while the roots of both plants remained undisturbed in the soil. All grafts were tightly wrapped with string and painted with grafting wax just hot enough to be liquid. Grafting wax is usually not necessary for stem grafts in plants of this kind, but if it is required, Murphy, and M'Kay (51) recommend the following preparation: Beeswax 4 parts; resin 2 parts; tallow 1 part. Harris (30) has described some grafting methods for investigating a suspected virus disease of the strawberry. Attempts to graft the crowns of strawberry plants failed, but successful grafts were made of the stolons. Two types of grafts were made: cleft grafting and inarching. The first method was similar to that used by Roach (61) for the grafting of potato stolons. Two runners in a similar stage of development were selected. The stock-runner was severed from its stolon and discarded, leaving the latter attached to the stock-plant. Two separate clove-hitches of artificial silk (loosely tied) were then slipped over this runnerless stolon. The scion-runner was then cut off so as to leave about 2 cm. of stolon at its base, and this length of stolon, held flat along the index finger of the left hand, was cut into wedge form by two slicing cuts with a safety-razor blade. The silk clove-hitches having been slipped down out of the way, the stock stolon was then cleft by a single cut with the razor-blade knife, the length of the cut corresponding to the length of the wedge on the scion-runner stolon. The latter was then introduced into the cleft, the clove-hitches were slipped up over the graft (one to the upper and one to the lower end) and were tightened sufficiently to hold it firmly without constricting it. Finally the graft was coated with melted paraffin wax and the surplus silk ends cut off. After grafting, the plants were kept in a humid atmosphere to prevent the wilting of the scion till union had taken place. The second method of grafting used by Harris was that of inarching: by this method it was found possible to graft a runner from one plant on to the stolon of another plant without completely detaching the runner from its parent plant till after union had taken place. The inarching was performed in two ways : in the first procedure two stolons, one from each of the plants to be inarched, with runners in a similar stage of development, were selected. The runner from the stock-plant was then severed from its stolon and discarded, and the tip of this stolon was cut into a wedge about 1·5 cm. long by two slicing cuts with a razor-blade knife. An oblique cut was then made with this knife on the stolon of the scion-runner, either just below that runner or about 8 cm. lower down. This cut was made starting towards the base of the stolon and working towards the runner, and was continued sufficiently deep just to avoid severing the vascular strand. The wedge-shaped tip of the stock-stolon was then inserted in the cut and the graft bound with raffia and waxed. In the second method of inarching used by Harris neither of the runners on the grafted stolons was removed. The procedure was similar to that used in the first method, except that an oblique cut was also made in the stock-stolon running in the opposite direction to that made in the scion-stolon. Before making these cuts a thin slice of epidermis and cortex the length of the proposed graft (about 2 cm.) was removed from each of the stolons. The oblique cuts were then made, and the tongues thus produced were engaged in one another, two cut surfaces of each runner (four in all) thus being brought into contact. Finally the graft was bound with raffia and the plants treated as by the previous method. After a lapse of ten to fifteen days the stolon of the scion-runner was severed just beyond the graft, leaving the stock-runner and the grafted scion-runner attached to the stock-plant by one stolon.

For studies on the virus diseases of the hop, grafting is necessary and Salmon and Ware (62) describe a method by which this can be accomplished. It has been found that the most suitable shoots are those having an internode of about $\frac{1}{8}$-in. diameter at 1 in. or so above soil level. Two or three pairs of leaves are expanded and the terminal bud is at a height of about 4 in. from the ground. The internode of suitable thickness is cut across with a sharp knife,

½ to ¾ in. above the node, and, for subsequent convenience in binding, at a height of 1 to 2 in. from the soil. Where it has been found necessary owing to considerations of height or thickness to cut off the stock closer to a node, no failure has resulted. With a razor, two downward cuts from opposite points on the periphery of the cut surface of the stem are made to converge in the centre of the pith at a depth of about ½ in., in such a way that a wedge-shaped piece of stem is removed. Any leaves below the cut are removed to prevent interference with the binding. The graft selected is the top of a similar shoot on another plant. If the tip of an elongated vine be chosen, it will be found that the cut end of the scion will tend to split and curl. Immediately the V-shaped cut has been made in the stock the selected tip is removed from the other plant with a sharp knife at a part of the internode at least ½ in. below the node above. It is then held with its base lying flat on a clean board, and the end is made wedge-shaped by means of two cuts ½ in. long.

With regard to binding material, raffia covered subsequently with paraffin wax (M.P. 45° C.) or with grafting wax or with a layer of damp cotton wool enclosed with tinfoil, has been used successfully. The use of very thin rubber tape in widths of ½ to ¾ in. as in the inner winding of certain golf balls has proved ideal for the purpose of grafting hops. Six inches of this are cut off and one turn is made in the winding around the stock, below the cut. The graft is then inserted and winding continued upwards to ¾ in. above the stock, the rubber being slightly stretched as it is wound. The winding is then continued downwards again until the starting point is reached, when the free end is simply secured by tucking it under the last coil or by the use of rubber solution. In this way the scion is held securely; no wilting results if the grafted shoot be immediately covered with a plugged lamp-glass or bell jar and sheltered from the sun for a week or ten days. Whip grafting and inarching have also been used successfully.

Guterman (29) in experiments on transmission of mosaic of *Lilium auratum* describes a method of inarching a healthy and mosaic plant. The lower leaves are removed, and by means of a razor a small slice is taken from the side of each stem. The cut surfaces are then quickly bound together with raffia and waxed with a mixture of paraffin and petroleum jelly.

Sreenivasaya (68) has used two methods for transmitting spike disease of sandal. The first of these is known as " patch grafting," and consists of the transfer of a piece of infected bark tissue, including the cortex and the bast, to a healthy stock. Three kinds of patches were employed: those with active buds, those with dormant buds, and those without buds. It was found that patches bearing actively growing buds were more virulent and transmitted the disease in a shorter time than those with dormant buds; patches completely free from buds were less infective than the other two varieties. The second method of transmission of spike of sandal described by Sreenivasaya is not, strictly speaking, grafting but consists of the insertion of a piece of infected leaf tissue under the bark of the healthy stock. A small " window " is cut in the stem leaving the flap of bark attached by one side; a rectangular piece of diseased leaf tissue is inserted between the wood and the bark and the flap of bark replaced over the leaf tissue. The whole is then firmly bandaged with wax cloth. If the operation is successful the injured portion fuses by callus formation in the course of ten to fifteen days and the leaf tissue becomes completely covered.

A method of tuber grafting for investigating virus diseases of the potato has been developed by Murphy and M'Kay (51). This procedure of " core grafting " consists in removing, by means of a cork-borer of about 13 mm. diameter, a core containing an eye from the tuber it is desired to infect, and inserting into the hole so made a core, removed with a cork-borer one size larger than the one first used, from the tuber destined to serve as the source of infection. The latter core may be taken without an eye at either end, and that from the healthy tuber may be taken with an eye and used to raise a plant to serve as a control. This method has also been used by Goss (27) to transmit the virus of potato spindle-tuber and by Young and Morris (79) for transmitting the virus of witch's broom of potatoes and tomatoes. A similar method of virus transmission by core grafting has been used by Bonazzi (8) in investigating mosaic of sugar cane.

Transmission by Inoculation

The second method of artificial virus transmission is that of *inoculation*, using this term in its restricted sense of actual application of the virus-containing sap to the plant tissue. Most of the mosaic group of viruses are transmissible by this means. The standard method of inoculation consists in triturating the tissue of the virus-diseased plant in a sterile mortar and then rubbing with the pestle the sap, thus extracted, on the leaves of the plant to be infected. There are various modifications of this method, which will be shortly described. Holmes (34) describes a method of inoculating test plants with small uniform doses of tobacco mosaic virus. By the use of small plants inoculated by pricks of insect pins held in a convenient handle, very large numbers of test inoculations may be performed with economy of time and effort, and with a minimum of greenhouse space and practically no danger of contamination from handling. Sein (65) has applied this method to the inoculation of mosaic of sugar cane, and he claims that it gives 94 per cent. of positive transmission. His method consists of stripping the central spindle of the outermost leaf, binding the cylinder of tender leaves thus exposed with a mosaic leaf and pricking through this with a number of insect pins mounted in a handle. This " multiple needle " method of inoculation has been applied by Dana (12) to the transmission of the virus of curly top of sugar beet, but the percentage of successful inoculation appears to have been low. This method, however, has given 100 per cent. transfer when used with a mosaic of cruciferous plants, which illustrates, as Dana remarks, the variable in facility with which the different plant viruses may be transmitted by mechanical methods. Holmes (34) has also shown that the most effective way of transferring mosaic virus to tobacco plants is a gentle rubbing over a large leaf surface with a cloth soaked in extract from mosaic plants. Scratches with a needle are much less effective even when made in the presence of the virus. The virus of tobacco mosaic does not readily enter wounds made in the leaves of healthy tobacco plants if these are made before its application. Entrance seems to be instantaneous upon the production of a suitable wound in the presence of the virus. Immediate removal of the excess of mosaic extract by washing does not decrease the total number of infections, but in some cases actually increases it. This last fact has some practical value when toxic solutions are employed in virus studies.

Petri (57) has evolved a special method for injecting mosaic virus into citrus leaves. Two metal discs are attached to the leaf by means of springs of the forceps type, the upper disc having fixed to it at an oblique angle a fine tube within which a slender steel needle can be inserted. The leaf is punctured by pressure of the button at the top of the needle, which is then withdrawn and immediately replaced by a sterilized glass capillary tube filled with the expressed infective juice. The latter may safely be left in the leaf for twenty-four to thirty-six hours without disturbing its physiological functions, so that the virus is absorbed gradually. Henderson Smith (32), in experiments with a mosaic disease of tomato, recommends that the leaf to be inoculated should be supported on the wooden label used to mark the pot; the inoculum is then dropped on the leaf and scratches made with a needle through the drops. This method avoids all contact of the hands with the leaf or inoculum, and is effective.

Bolas (7) describes a modification of the method of inoculation used by Holmes (34) which consists of gentle friction with cheese-cloth impregnated with virus sap. The method used by Bolas consists in tearing and folding a small portion of clean dry filter paper by means of sterile forceps, moistening the edge of the folded paper with the inoculum and applying the moist edge to the leaf of the plant to be infected with just sufficient friction to produce the slightest mechanical injury. Bolas claims that this method is very much quicker than the method of pricking through a drop of inoculum on the leaf and gives a much higher percentage of infections. Other methods of inoculation with the virus of tomato aucuba mosaic may be mentioned; these are pricking through a drop of infected sap placed on the leaf to be inoculated, scarification with a roughened glass scraper moistened with infected sap, and gentle friction with the infected leaf after cutting and tearing in order to preak some of the cells of the infected leaf, moistening it if necessary. Bolas considers that the friction with infected filter paper gives 15 per cent. more infections than any of these other methods of inoculation. Samuel (63) uses flat glass spatulas for general inoculation work. These are made as follows: after pressing out the expanded portion to a width of about 1 cm. and at an angle of about 135° to the handle, the underside is ground flat on a carborundum wheel or on carborundum paper. This gives a very good rubbing surface, which in comparative experiments yielded results as good as, or even slightly superior to, those produced by the muslin wiping method. The leaf which is being rubbed with the spatula dipped in virus is supported by the left hand, the fingers of which are protected from contamination by a small square of clean paraffin paper held under the leaf. The experiments carried out by Samuel show that the viruses of the following diseases are more effectively transmitted to tobacco by a light rubbing of the leaves without visible wounding than by needle scratches—tobacco mosaic, cucumber mosaic, spot-necrosis of tobacco, ring spot of tobacco, Petunia mosaic, yellow tobacco mosaic, and spotted wilt of tomato. Jones (39) suggests a method of inoculation in which round swab sticks or " applicators " similar to those used by the medical profession are employed. These are broken in half and absorbent cotton is firmly rolled on to one end of each half applicator and securely fastened with thread. In making inoculations from growing plants, portions of the plant tissue are broken off with one of these sterilised swabs and a sterilised pot label. The plant tissue is macerated on the label and then rubbed over the foliage to be inoculated by means of the swab. The pot label acts as a support of the leaf being inoculated. It is claimed that by using care neither the tissue used as inoculum nor the plant to be inoculated comes in contact with any source of contamination.

McKinney (46), in describing methods in virus studies with tobacco mosaic, recommends the insertion of a piece of absorbent cotton lint, dipped in virus extract, into the vascular region of the stem and petiole.

Johnson and Duggar (35) claim to have produced 80 per cent. transmission with " typical " tobacco mosaic by stomatal infection. Their method was to spray the healthy plants with virus extract by means of an atomizer. It is difficult to reconcile these results of Johnson and Duggar with the statements of Caldwell: first, that the exudate from the hydathode does not contain virus (10), and, secondly (11), that he has injected the intercellular spaces of the leaf of *Nicotiana glutinosa* with virus juice and no infection took place unless cells were accidentally punctured.

In a few cases the more infectious plant viruses can be disseminated by means of the cutting or pruning knife in the ordinary course of tending the plants (78).

Transmission by Seed

The next method of virus dissemination to be discussed is transmission by the *seed*. This is not of common occurrence, but there are several authentic cases, the best known being mosaic of bean, *Phaseolus vulgaris*. In commercial seed of susceptible varieties as high as 50 per cent. infection may sometimes develop (20). The percentage of infection of the bean seeds is, however, very irregular, varying from 13 to 50 per cent., and not all of the seeds in one pod may be infected. Ray Nelson (53) considers that an explanation of these irregularities in seed transmission and in localization of symptoms may possibly be found in the assumption that the virus is restricted to certain tissues, and that localization of infection prevents or retards dissemination of the causal agent to all portions of the plant. Further, supposing that the virus of bean mosaic moves through the vascular tissue, the failure of some seeds to become infected with the virus can be explained by the type of arrangement that characterizes the vascular tissues of the pod. The position of the seed in the pod does not determine whether or not it will receive the virus; the chances are about equal that seeds in any position may escape infection. It is probably not correct to assume that all viruses are uniformly distributed throughout the plant, and there is a certain amount of evidence that in certain cases the virus may become localized in the plant, resulting in a few foci of infection only. This question is further discussed in Chapter VII., p. 178.

The first record of seed transmission of the mosaic disease in leguminous plants was given by McClintock (43) in the lima bean. Further instances of the transmission of the virus of bean mosaic by the seed will be found in the work of Reddick and Stewart (60), Kendrick and Gardner (40), Burkholder and Müller (9A), Merkel (49), and Pierce and Hungerford (58).

As regards seed transmission of the viruses affecting Solanaceous plants, the position is not very clear considering the economic importance of the question. Bewley and Corbett (5) state that accurate proof of seed transmission of tomato mosaic long defied all attempts to secure it by the standard methods, but that recently 6·04 per cent. of infected seedlings have been obtained from a batch of 493 tomatoes grown from seeds taken from infected fruits. Bewley and Corbett offer as further evidence of seed transmission the fact that they have infected healthy tomato plants with the crushed embryos of seeds taken from infected plants. Immature seeds were taken out of the unripe fruit of a tomato plant severely infected with " aucuba " mosaic. The seeds were first washed in 80 per cent. alcohol for fifteen minutes and then in several changes of distilled water. After thorough washing, the embryos were dissected from the seeds and washed in several changes of sterile distilled water. An inoculum was prepared by crushing in a little distilled water, and tomato plants were inoculated. Of the six plants inoculated four became infected ; in a second experiment four plants out of twenty-four developed mosaic. That this experiment does not prove that these infected seeds would necessarily give rise to infected plants is shown by a parallel experiment carried out independently by the writer. Seeds were dissected out of berries from the black nightshade (*Solanum nigrum*) infected with the " Y " potato virus (67), washed for two hours under the tap, and then inoculated in a paste to tobacco seedlings. A high percentage of infection resulted ; yet the writer has never had a case of an infected *Solanum nigrum* seedling arising from seeds taken from infective berries, although it cannot be said that such a thing never occurs. It seems possible for the virus to be either adsorbed to the outside of the seed coat or contained within the seed coat, and yet for the seed to give rise to a healthy plant. Incidentally, washing the seed from tomato affected with " aucuba " mosaic with 80 per cent. alcohol, as in the experiment of Bewley and Corbett, would not remove the virus, as, according to Henderson Smith (32), it is not inactivated by alcohol below a strength of 90 per cent. A further experiment carried out by the writer on the seed transmission of the " Y " virus in *Solanum nigrum* may be mentioned here. A batch of seed from ripe infected berries was divided into two portions, one portion washed under the tap for two to four hours, while the remainder was stored for some weeks in an envelope at room temperature. After the given period of washing under the tap, the first lot of seed was ground up in a mortar and the resulting paste inoculated to healthy tobacco seedlings : this gave a fairly high percentage of infected plants. After a period of some weeks the other portion of the same lot of seed, now completely dried, was also ground up in a mortar and inoculated similarly to tobacco : these inoculations were negative in every case. This experiment seems to indicate that the virus " Y " was either adsorbed to the outside of the seed and could not be removed by washing or was enclosed within the seed coat and was inactivated by desiccation. The criticism may be made that such drying of the seeds is unlikely to occur under natural conditions and that infected seeds which have remained damp might give rise to infected plants. This point has been tested to a limited extent by planting numbers of seeds directly after they had been extracted from the ripe infected berries, but no infected seedlings were produced. Duggar, in a paper on the problem of seed transmission of the " typical " tobacco mosaic (18), draws attention to the probability of the relation of transmission to adsorption and inactivation through storage proteins. It is perhaps of interest in this connection to mention that the juices of *ripe* tomato fruits appear to inactivate the virus of tomato spotted wilt, which would preclude the possibility of transmission of this virus by seed of infected tomato (3, 66). Possibly some such phenomenon is concerned with the seed transmission of a similar virus—tobacco ringspot—which is apparently carried by seed of Petunia but not by the seed of other hosts (31). Berkeley (4) offers evidence of seed transmission of tomato " streak " in Canada. It is not clear what virus disease is indicated by " streak," possibly it is the same as that known in this country as tomato " stripe." " Streak " of tomato in Europe is considered to be a virus complex. As regards the seed transmission of the economically important potato viruses, little work seems to have been carried out on the subject. Murphy and M'Kay (50) are of the opinion that potato leaf roll and some of the potato viruses of the mosaic type are transmitted through the true seed. Experience seems to indicate that the presence of occasional plants infected through the true seed is not unknown in commercial establishments devoted to the raising of new potato varieties.

Elze (19) considers that he has evidence that leaf roll and " aucuba " mosaic of the potato are carried in the seed. The study of this problem in the potato plant, however, is complicated by the distortions, mottlings, and virus-disease-like symptoms which develop in potato seedlings, due apparently to chromosome abnormalities.

Bewley and Corbett (5) offer evidence of the transmission of the virus of cucumber mosaic by the seed ; Doolittle (15) considers that this virus is not transmitted by the seed of the cultivated cucumber, but that it is so transmitted in the case of wild cucumber, *Micrampelis lobata* (16). It is questionable, however, whether mosaic of cucumber in the two continents is due to the same virus.

The virus that produces variegation in *Abutilon Thompsonii* and *Abutilon mulleri* is in a limited way transmissible to some of the seedlings obtained by crossing these two clones. In an experiment involving 536 seedlings obtained from this cross, 287 were green and 249 showed " variegated " characteristics, which could be transmitted in but four instances to susceptible green *Abutilon reguellii* scions by grafting (41).

It is known that the virus of bean mosaic is transmitted irregularly through the seed, and that not more than one-half of the seeds are infected in pods harvested from plants arising from diseased seed. To test the possibility of pollen infection and to determine the relative effects of pollen and ovule infection in seed transmission of the disease, crosses were made between Refugee and Early Prolific, varieties very susceptible to mosaic. The incidence of mosaic in hybrids between mosaic Refugee and healthy Early Prolific was approximately the same as in hybrids between healthy Refugee and mosaic Prolific. The mosaic parent in all crosses was produced from infected seed. The results indicate that about one-quarter of the ovules and pollen grains are infected with virus. In crosses between Refugee and Robust, the latter being a highly resistant variety, about one-quarter of the hybrid seedlings were severely affected with mosaic (54).

The following is a list of some other plant viruses which are considered to be transmitted through the seed : mosaic of *Dolichos biflorus* (Uppal (70)) ; mosaic of beet (Ducomet (17)) ; a tobacco ringspot by seed of Turkish tobacco (Johnson, E. M. (36)) ; mosaic by seed of *Pisum sativum*, *Trifolium pratense*, *Trifolium hybridum*, *Melilotus alba*, and *Hippeastrum* sp. (Dickson (14)) ; " dot " mosaic by seed of lettuce (Brandenburg (9), Newhall (55)) ; two ringspot diseases of tobacco (Valleau (71)), and possibly rosette of wheat (McKinney (45)). The reader should also consult a useful bibliography by Orton (56) on seed-borne parasites.

As pointed out by Ray Nelson (53), it is rather remarkable how little investigation has been carried out upon this question of the transmission of plant viruses by seed, and it is probable that research upon this question would yield interesting results.

Transmission by Pollen

The next mode of virus transmission is by the pollen, and on this subject there is very little information. Blakeslee (6) considers that a mosaic virus affecting *Datura stramonium* is transmitted through pollen to the seed of 79 per cent. of the offspring. The only other case appears to be that described by Reddick (59), who offers evidence that the virus of bean mosaic is transmitted by this method. Transmission by the pollen has been suggested on more than one occasion in order to explain the spread of certain virus diseases for which no insect vector can be discovered, notably " spike " of sandal and others. Pollen transmission would explain some points in the spread of certain potato viruses of the " X "-type which are almost universal in commercial potato crops but which do not appear to be transmitted by the aphides and other common insect fauna of the potato. There is, however, no evidence as yet of such a mode of transmission of these viruses.

Transmission by Contact of Foliage and Roots

As regards transmission of viruses by contact of foliage and roots, it must be borne in mind that a wound of some kind is essential for entrance of the virus even if it consists only in the breaking of a trichome. It is probable that a certain amount of transmission takes place in this way among the more infectious viruses of the tobacco mosaic type, particularly in glasshouses where tomato plants may be growing close to one another. Similarly in tobacco fields, high winds and storms may play a larger part in the dissemination of tobacco mosaic than is realized.

McWhorter (48) claims to have demonstrated experimentally that mosaic of narcissus, the so-called narcissus " gray disease," known in England as " narcissus stripe," is transmissible by accidental damage to the roots. " Golden Spur " bulbs of diseased and mosaic-free stocks were planted in 1930 so that diseased and healthy plants were interspersed in the rows. The mosaic plants were dug out in 1931. In one of the test rows this roguing process exposed 62 adjoining plants to possible mosaic transmission by mutilating the roots. Of these 62 plants so exposed, 16 plants actually developed the disease in 1932. In other words, 25 per cent. of the plants adjoining those which were rogued developed mosaic. McWhorter considers that this is a case of root transmission of a virus made possible by injury accompanying roguing.

McKay and Dykstra (44) consider that the potato virus disease rugose mosaic is transmitted, presumably by contact alone, from diseased to healthy plants under insect-proof cages in the absence of aphides and leaf or root mutilation. They also find that this disease can be transmitted by root mutilation induced by methods corresponding to the ordinary routine of potato cultivation. Schultz and Folsom (64) were unable to transmit spindle-tuber and leaf roll of potato by contact of roots and foliage in the absence of insects, but they report that mosaic was transmitted by this means. In the writer's opinion further confirmation is needed on this question of transmission of potato viruses by contact alone.

McKinney (46) finds that soil transmission of tobacco mosaic takes place in the absence of all soil fauna ; and Webb (73), working on mosaic of wheat, states that infection occurs through either the roots or the crown of the plant or both : the possibility of transmission by insect vectors, however, does not appear to be excluded in this latter case.

A source of infection to tobacco fields which may conveniently be mentioned here is the dissemination of the mosaic virus by workers who " chew " home-grown tobacco. The results of observation indicate that the grower who " chews " can carry infection into the seed bed, when picking or weeding seed beds or drawing plants, by spitting on the seedlings, handling the leaf, or wiping the mouth with the hands, followed by bruising of plants in handling and tramping (47). The risks of mosaic infection to tobacco fields arising from the worker's habits of chewing and cigarette smoking have been further emphasized by Valleau and Johnson (72), who found that sixteen out of forty-five commercial brands of tobacco tested gave positive infections in inoculation tests. Similarly Fukushi in Japan obtained infection from twenty-five packages out of thirty, comprising four commercial brands of cut tobacco (24).

Factors Governing the Spread of Certain Plant Viruses in the Field

There are two main factors which govern the spread of plant viruses, and these will be discussed shortly. The first factor concerns the migratory movements of the particular insect vector or vectors concerned, and the second, in the case of annual crops, is the means by which the virus survives the winter. The spread of virus diseases which are dependent upon a specific insect vector, or groups of insect vectors, for their transmission, naturally follows the movements of such insects, although other factors are involved. An investigation, therefore, into the spread of a virus resolves itself largely into a study of the behaviour of the insect vector in its natural habitat. Some points concerning the spread of potato virus diseases in the field and the movements of the chief aphis vector will be considered first. Experiments in Canada on the spread of leaf roll (52) indicate that in one season the virus spreads very considerably to the rows of healthy plants adjoining the row of leaf roll plants, and in progressively smaller amounts to the outer rows. Similarly in Indiana it was found in regard to leaf roll that the percentage of seasonal infection decreases with increasing distance from the source of leaf roll infection, and it would appear that there is not much danger of the disease spreading from one field to another if the latter are well separated (26). According to Folsom (21), leaf roll spreads more in Southern Maine, U.S.A., than in the northern part, sometimes spreading 900 feet. Mild mosaic increases enough in Northern Maine to be serious. Apparently the climate to the north and east is progressively less favourable to the spread of these diseases.

Werner (75, 76) has made observations on the rate of spread of the potato virus disease known as "spindle-tuber." He found that the disease was transmitted across several rows of plants, but that most infection occurred in the rows nearby. As the distance from the source of spindle-tuber infection decreased, the percentage and severity of the infection increased, but it was still very severe in the sixth row.

In Maine, U.S.A., in 1922, thirty-five fields were sampled in a study of the amount and distance of spread of mosaic and spindle-tuber from diseased to healthy fields. The samples, grown in 1923, showed that in 1922 one or both diseases had spread, in some places having done so enough to infect half the plants in the first five rows next to diseased rows, and in other places spreading across the healthy field as far as the fortieth and the fiftieth row. Plant-to-plant spread in a partly diseased field was also found to be common. On the other hand, in some places isolated, healthy fields, and even healthy fields grown next to diseased stocks, remained mostly or wholly as healthy as before. In 1923, thirty-six fields were sampled. The samples grown in 1924 showed that in 1923 one or both diseases often had spread, in some places infecting a third of the plants in the first five healthy rows next to diseased rows, and in some other places spreading across the healthy field as far as the fiftieth row. A general increase of disease due to spread from one plant to another in partly diseased stock was disclosed as being more frequent and as being probably greater in effect than the spread from one field to another. Therefore the secondary effects of field-to-field spread may be worse than the immediate effects. For some reasons unknown, these two diseases did not increase or spread in some instances where conditions seemed favourable. They also did not spread in most of the fields where there was some isolation from diseased stock. Some data are available on the direction of spread of these two viruses in three fields. In one case, spindle-tuber spread somewhat from the diseased field on the north side, but not from the one on the south side ; here the former was much more diseased, and was of a variety that matured earlier, thus inducing earlier dispersal of insect vectors. In another field, where fifty-one rows of healthy stock were planted between two parts of the same diseased stock, the spread southward from the diseased stock on the north side was much more pronounced, although the plant-to-plant spread was no greater on the north side. It was also found in a third field that the plant-to-plant spread of both mosaic and spindle-tuber was greater from the south-east to the north-west. An explanation for this is suggested by the fact that in 1923 in the same field, planted with similar stock, an increasing degree of infestation of aphides from the south-east to the north-west was found, this indicating what may have occurred in 1922 (22, 23). Goss (28) has studied the rate of spread of potato virus diseases in Western Nebraska, and he finds that mild and rugose mosaic spread slightly in two out of the four years of the experiment, and their spread could be correlated with the occurrence of aphides. Leaf roll spread only to a distance of about three plants from the source of infection, while spindle-tuber was transmitted more extensively, to the extent of 42 per cent. after four years. Whitehead *et al.* (77) have made a study of the aphis population on potato crops in various centres of Wales, and they have drawn the following conclusions. The absence of increase of virus diseases, and of leaf roll in particular, at the more successful centres, is not due to the scarcity of aphides, nor to the absence of known vectors of the viruses, such as *Myzus persicæ*. Neither can it be attributed to the non-infective conditions of the aphid vectors present at these centres, since representative samples, taken from potato crops, transmitted leaf roll to healthy plants under glass. With one exception no transmission occurred with any of the species of aphides when taken from apparently healthy plants in a partially diseased crop. In aphis samples taken from leaf roll plants transmission only occurred when they included *M. persicæ*. The accumulated data suggest that the maintenance of health in potato crops is influenced not merely by the relative abundance of aphides, but rather by the relation between the date of maximum infestation and the stage of maturity of the foliage. The more successful centres, each year, showed either a delayed maximum infestation and/or the foliage was cut down earlier than at the less successful centres. The relative movement of aphides within the crops at the different centres is certainly of importance. Ecological studies of aphides affecting potato crops have been made by Davies in North Wales (13), who devised a technique for ascertaining the movement of aphides within the crop. Weekly records on observational leaves revealed that 100 per cent. of the aphides moved their site, and at least 84 per cent. of each species moved from leaf to leaf within the weekly period. Daily records during the period of maximum infestation showed that 84 per cent. of *Macrosiphum gei* Koch, and 73 per cent. of *Myzus persicæ* Sulz, changed site within twenty-four hours, and of these at least 50 per cent. moved from the leaves upon which they had been observed. It seems clear, therefore, that the aphides move freely enough about the crop. As regards the relationship between maximum aphis infestation and maturity of foliage, Davies found that maximum infestation was reached during mid-July, when 86 per cent. of the leaves were infected and there was an average of 2·8 aphides per leaf. *Macrosiphum gei* bred up rapidly, at first on the flower heads, and was the predominant species, its decline in numbers being equally rapid. *Myzus persicæ* increased more slowly and did not reach such numbers, but the maximum was maintained longer and the disappearance of the species was delayed. *Myzus pseudosolani* Theob. was only taken in August, and then in small numbers. The importance of *Macrosiphum gei* in relation to potato virus diseases is problematical, and, of the three insects, *Myzus persicæ* is the significant factor. Davies attempted an estimate of the intensity of the aphis population, and considered that a moderate infestation of 2·8 aphides to a leaf was of the order of 2,000,000 aphides per acre of potatoes. Aphis counts on potatoes have been previously made by Folsom and Schultz (22), who estimated from the average numbers on five-leaf samples that the population averaged about 1,000 aphides per plant, and 1,000 aphides per plant might be expected to result in considerable spread of virus. There are, however, as the writers have pointed out, other considerations—first, a number of these aphides are winged and are the autumn migrants about to seek plant hosts other than the potato ; and, secondly, such high infestation of aphides usually only occurs when the potato plants are practically mature and when they are difficult to infect with virus diseases.

An ecological study which is urgently needed is a comparison of the aphis species and population of the Scotch " seed " potato districts with representative districts in England and Wales. Such a study might throw light upon the comparative freedom of the Scotch " seed " from virus infection as compared with English " seed " and indicate whether this freedom from virus infection could be correlated with the absence or scarcity of the chief vector of potato viruses, *M. persicæ*. A survey of this kind was commenced in 1931 by the writer's colleague, Mr. J. P. Doncaster, who toured the Scotch " seed " potato districts in Ayrshire and Aberdeenshire during July and made observations on the aphis species occurring there. Unfortunately, owing to financial stringency, the survey had to be temporarily discontinued in 1932. Doncaster's preliminary notes are given herewith.

The aphis *M. persicæ* was found in small numbers on experimental plots at Corstorphine, Edinburgh, averaging about 0·5 per plant. Similar infestations of aphides were observed in " seed "-growing (Epicure) areas in Ayrshire. Here, however, the chief species was *Macrosiphum gei*, and very few specimens of *M. persicæ* were observed. Three days' observations in Aberdeenshire (Huntley and Aberdeen district) revealed no aphis infestation on potatoes whatever. So far as these observations go, therefore, they indicate a scarcity of the virus-transmitting aphis *M. persicæ* in the Scotch " seed "-potato districts.

As regards mosaic of beets, an aphis-transmitted virus, it has been shown that the closer seedling beets are grown to infected mother beets the greater is the spread of the disease to the seedlings. The disease has been observed to be transmitted sparingly, at least one and a half miles from infected plantings (38).

Some studies have been carried out in Idaho on the movements of the beet leaf hopper in relation to incidence of the virus disease curly top. By June 17th–22nd the leaf hopper populations were extremely high in the sections concurrently showing a large percentage of curly top, and by August 11th leaf hoppers had infested 90–95 per cent. of the crop of Southern Idaho. The general movement of the insects was from the north-west and west (1). It is claimed that the problem of controlling the leaf hopper vector of curly top (*Eutettix tenellus*) has been solved by an organized campaign of destruction against the Russian thistle, *Salsola kali*, a favourite food plant of the insect, combined with extensive use of an oil-pyrethrum spray (2).

The problem of how plant viruses attacking annual crops are carried over the winter is largely, but not entirely, a question of host range and whether the virus is able to affect perennial plants or plants propagated by cuttings. The question of the survival and transmission of plant viruses in the seed of affected plants has been dealt with on p. 197. Viruses affecting perennial plants persist, of course, from year to year, either in the plants themselves or in their perennial organs, such as root-stocks, rhizomes, tubers, and bulbs. The economically important group of potato viruses fall into this category.

A good illustration of how a virus affecting an annual crop passes the winter is afforded by the disease attacking tomatoes known as spotted wilt. The virus causing this disease has a wide host range and affects, among others, two plants which are propagated almost entirely by cuttings, *i.e.*, *Solanum capsicastrum*, or Orange Flower, a plant much grown in this country for winter table decoration, and a type of double nasturtium, which must be propagated by cuttings as it does not set seed. The spotted wilt virus will also attack dahlias and thus persists in the tubers of that host.

Leaf curl of cotton is a serious disease in the Sudan and elsewhere, and the question of how the virus survives the hot weather in the absence of the cotton crop is one of considerable importance. There seem to be three possible methods by which the virus might be carried over this " dead " season. First, by the aestivation of virus-infected whiteflies—this is not very probable ; secondly, by persistence in other plant hosts, of which the most likely seems to be the garden hollyhock ; and, thirdly, in the ratoon cotton plants which survive the dead season and produce new growth—which appears to be almost always diseased—when the land which had been previously under cotton is irrigated in August. This last is considered to be the main method by which the virus is carried over (42).

The virus of beet mosaic has been shown to over-winter in the beet roots in the pits, and such infected mother beets act as a source of infection when planted in the field the following spring (38). The weed *Commelina nudiflora* harbours the virus of celery mosaic in Florida and so acts as a reservoir of infection (74). Similarly, in Indiana tomato mosaic persists in certain perennial Solanaceous weeds such as *Physalis subglabrata* and *Solanum carolinense* (25). The virus of tobacco mosaic has been shown by Johnson and Ogden (37) to exist for long periods of time in pieces of roots and in dead plant material in the soil, and this is considered to be a common source of infection to new crops of tobacco in the following spring. Some factors which appear to be connected with the spread or incidence of " spike " disease of sandal are of interest. These factors, the true significance of which is not yet understood, have been discussed by Sreenivasaya and Rangaswami (69). The differences found between healthy and spike-diseased areas are very striking and suggestive. Diseased areas are characterised by a complete absence or diminished occurrence of certain types of flora, like *Barleria buxifolia, Cippedessa fructicosa, Dodonea viscosa, Breynia rhamnoides,* etc., while the preponderance of certain species of plants in healthy areas lends significant support to the theory that disease resistance in the sandal plant is controlled by particular types of host plant. The high percentage of coppiced and dead stumps of host plants found in diseased areas is significant and supports the observation made in the laboratory that infected sandal succumbs to the disease more rapidly in absence of a host plant.

The frequent association of abundant Lantana with diseased areas led to the belief that it had some bearing on disease incidence. The rapid spread of Lantana in a given forest area is invariably the result of either indiscriminate exploitation of the stock or frequent and severe fires, both of which result in opening out the forest and favouring the growth of Lantana. In other words, the sandal stock in the area suffers a change from deep-rooted and sturdy host trees to surface-feeding bushes of doubtful host value, like Lantana, *Acalypa indica,* and others.

One of the most striking observations is the constant association of agricultural operations with the primary site of the first disease attack. Artificial interference with virgin areas by cutting down the host plants associated with sandal has been responsible for rendering it susceptible to disease, new outbreaks of which have always taken place in areas close to agricultural fields or colonies.

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CHAPTER IX

PHYSIOLOGY OF VIRUS DISEASES OF PLANTS

Movement of virus in the plant host—Local lesions—Metabolism of virus-affected plants—Metabolism of viruses.

Movement of Virus in the Plant Host

THE question of the translocation or movement of the viruses within the plant has recently been reviewed by Henderson Smith (17), and some of his conclusions are given here; work on this subject which has appeared since that review will be discussed subsequently.

There are at least two obvious routes along which the spread of the virus may be effected. First, the virus may spread from cell to cell by diffusion; that this does occur is shown by the behaviour of the virus of tobacco mosaic which may be introduced into the leaf by stroking with an infected camel-hair brush only sufficiently hard to break a few trichomes, and in this case the virus must pass through the cell walls to reach the leaf tissues. Diffusion from cell to cell, therefore, is in all probability one of the modes of spread, but it is not yet known which cells are involved in the movement of virus in the systemic infection. This method of spread must be, as Henderson Smith has pointed out, slow in the case of viruses which can be held back by certain membranes. The second possibility is movement of the virus in the vascular bundles either in the xylem or the phloem, or both. It will be suggested later that the xylem vessels are not normally involved in the dissemination of the virus agent. Holmes (21), investigating the direction of movement of the virus of tobacco mosaic in young tobacco plants, states that virus was found, from the time of the appearance of the earliest symptoms until the end of three weeks from the time of inoculation, to be present in large amounts in the inoculated leaf and those above it, and to be absent from the leaves immediately below. The later development of virus in the lower leaves must indicate a migration of at least a small amount of virus backward down the plant. Böning (7) came to the opposite conclusion. He found, also in tobacco, that on testing the tips of the leaves immediately above and below the inoculated leaf, the virus was first present sometimes in the upper and sometimes in the lower leaf, but that both leaves showed symptoms on the same day. Grainger (16) considers that the virus travels from the base of the stem first to the growing point and young leaves. It then multiplies there and spreads to the progressively older leaves in the inverse order of their formation. Henderson Smith concludes that in general virus moves in either direction in the leaf, and that in the stem, even if the upward route is more usual—which is not established—downward transmissions can on occasions occur at least as rapidly, and movement in both directions may occur in the same plant at the same time.

Bennett (5) has studied the movement of the curl virus in raspberry plants by means of girdling experiments. If a plant were girdled completely, virus did not pass the girdle in either direction, but if even a small bridge was left across the girdle, the virus passed across. Bennett's conclusion was that transport took place in the channels which conveyed elaborated food material, the movement of the curl virus appearing to show evidence of a correlation with food translocation.

Some experiments on the movement of the virus of " aucuba " or yellow mosaic in the tomato plant have been described by Caldwell (10). In the test plant a middle portion of the stem was killed either by steam or chloroform, leaving a bridge of dead tissue between the upper and lower portions of the plant, and inoculations were made either below or above this bridge (see Fig. 35). In neither case did the disease develop in the portion of the plant on the other side of the bridge. The results, which indicated that the virus agent was not travelling in the xylem stream, lead Caldwell to believe that translocation of the " aucuba " mosaic virus in the tomato plant takes place in the living ground tissue and in the phloem. In a later paper Caldwell (11) states that normally the virus agent does not enter the water stream of the plants, but, if introduced experiment-

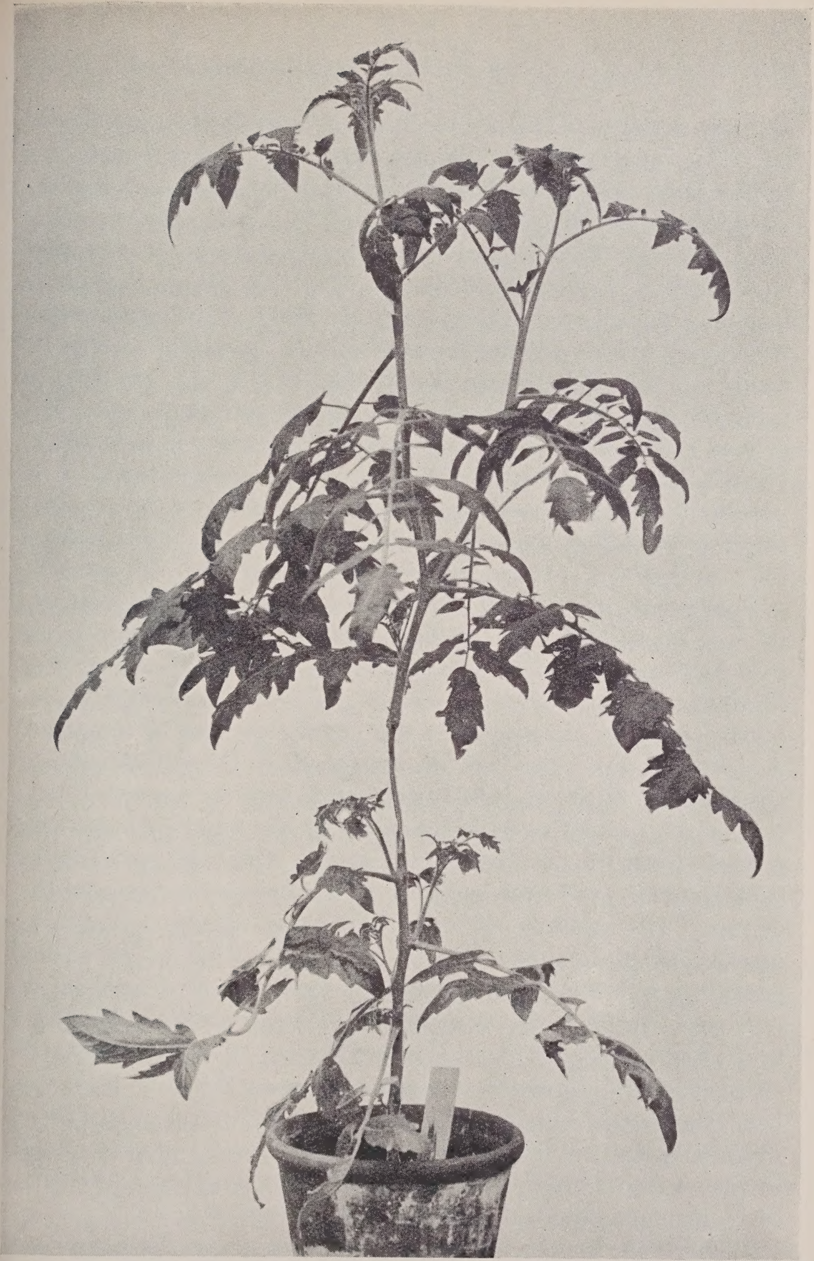


FIG. 35.—Tomato plant in which the stem has been steamed at one internode. Subsequent to inoculation, below the steamed area, with the virus of yellow or "aucuba" mosaic, symptoms appeared on the lower part of the plant, while the upper part remained healthy. (After Caldwell.)

ally, it is carried round in the xylem vessels, from which it cannot pass into the ground tissues and phloem unless the vessels are mechanically injured. All the indications are that the agent cannot enter an unbroken cell or move into or out of dead cells. On the other hand, Grainger (16) has arrived at the opposite conclusion, and states that the virus of tobacco mosaic will move either up or down across a steamed area in the stem. He also found that the virus would move across a steamed area in a leaf. It should perhaps be mentioned that Caldwell has also performed the same experiment using the virus of tobacco mosaic in the tobacco plant, and again arrived at the same conclusion as with " aucuba " mosaic of the tomato, *i.e.*, that the virus did not cross the steamed area. It is possible that in Grainger's experiments the whole of the steamed ring was not killed, and this would account for the virus passing the " bridge " of steamed tissue.

Holmes (22) has studied the local and systemic increase of the tobacco mosaic virus in the host. The mosaic virus developed to a high concentration near the site of inoculation in a leaf of *Nicotiana tabacum* before reaching measurable concentration in other portions of the inoculated leaf or in other parts of the plant. A slow spread of virus through the tissues of the inoculated leaf accompanied the increase in concentration near the site of inoculation, and appeared to be independent of the rapid spread which carried virus to distant parts of the plant. This local increase and slow spread of the virus constitute a local or primary phase of the disease. The systemic or secondary phase of the disease was marked by the nearly simultaneous appearance of increasing quantities of virus in the petiole of the inoculated leaf, in all portions of the stem, in the developing top leaves of the plant, and in the roots with later invasion of old leaves. In a series of plants all successfully inoculated in similar leaves at the same time, the local increase of virus within the tissues of the inoculated leaf blade occurred simultaneously in all plants, but systemic spread of virus with its attendant mottling of developing leaves occurred early in some individual plants and late in others.

Holmes has recently carried out further studies on the movement of mosaic virus from primary lesions in *Nicotiana tabacum* (23), in which he describes three main lines of work : (1) the application of the iodine technique (see below) in studying the path taken by the virus in early stages of systemic infection of *Nicotiana tabacum ;* (2) the modification in the path of the virus in leaves of experimentally treated plants ; (3) some factors influencing the time required for the virus to reach, and produce symptoms on, the leaves at the top of the plant. The results of these experiments show that the average interval between time of inoculation and appearance of systemic symptoms is shorter when the number of primary lesions is large than when the number of primary lesions is small ; and when the inoculation is near the base of a leaf than when it is near the apex of a leaf. The extension of primary lesions along large veins in uncut inoculated leaves appeared to indicate that large veins were important for movement of virus from the area of inoculation. The affected areas around cuts appeared more extensive than the affected areas in uncut leaves. It appeared probable that the virus was carried rapidly round the cuts as though by some diverted stream through small veins and into parts of the leaf which were not ordinarily so promptly invaded. In none of the cut leaves did severe wilting of partly isolated areas occur ; it was therefore evident that water could move round the cuts to distant parts of the plant. The failure of the virus infection to follow this water movement and its abnormally rapid extension in the opposite direction agrees with Caldwell's contention that the virus does not move in the xylem (see above). Storey (55) has also found that, in the case of the virus causing streak disease of maize, movement of the virus was not markedly delayed by cutting out the midrib of the leaf or cutting across the half-lamina on which the inoculation was made.

Holmes has demonstrated that a change in the path affected by the virus in the plant can be secured by shading the inoculated leaf (23). A great many one-sided infections of the inoculated plants appeared, and a large proportion of the leaves showing clearing of the veins also showed one-sided infection as manifested by unsymmetrical patterns shown up by the iodine-staining technique. The following experiment was performed : a set of 120 plants was inoculated on one leaf of each plant, and the set was then subdivided. In one-third of the set complete shading of the inoculated leaf was ensured by covering it with tinfoil, but the remainder of the mature or nearly mature leaves of the plant were left exposed to the light. In another third of the set the inoculated leaf was left uncovered, but two mature or nearly mature leaves near the level of the inoculated leaf but on the other side of the stem were covered with tinfoil. In the remaining third of the set no leaves were covered. The plants with covered inoculated leaves showed eighty-four leaves with clearing of the veins, and of those forty-eight showed unsymmetrical patterns with iodine staining. The plants with two covered inoculated leaves showed 109 leaves with clearing of veins, but none of these showed such patterns. The plants with no covered leaves developed clearing of the veins on 117 leaves ; of these thirteen showed one-sided infections. This experiment suggests the possibility that the virus may be carried with exports of food material from leaves, or by the mechanisms ordinarily serving to transport such material. Holmes also found that there was an earlier spread of virus to the top of an old plant when all leaves but the one inoculated and young leaves near the growing point were removed, and this suggests that the inoculated leaf under those circumstances may supply more food to the dependent leaves at the top and that virus may go with this food or be influenced in its spread by changed conditions in the tissues. Grainger (16), however, considers that the movement of the virus in the host seems to show potential independence of any help from the host in so far as the movements of food and water in the plant can be compared.

Finally, there appears to be some interference with free and uniform movement of the virus within the leaf tissues. The evidence seems insufficient to determine whether the interference with virus movement is of the nature (1) of interference with customary speed of movement of virus within cells, as by decrease of protoplasmic streaming or by decrease of translocation of food materials ; or (2) of the nature of interference with movement of virus from one cell to another, as from some restriction of the passage through cell walls ; or (3) from some modification of the permeability of the cell membranes. Holmes considers that this interference with virus movement may throw light upon (1) the nature of those hosts which localize the virus, such as *Nicotiana glutinosa* and *Datura stramonium ;* (2) may lead to a better understanding of the nature of symptomless carriers ; and (3) may possibly furnish information concerning some phase of the nature of the virus itself, since the virus appears to possess a property so adjusted to the character of the cells of the plant in which it is propagated that the virus can pass from cell to cell but meets opposition and a measure of delay in this passage.

Rate of Movement of Virus. The *rate* of movement of viruses within the plant host has been measured in a number of cases; Allard (1) found that three days elapsed before mosaic virus, inoculated at the tip of a tobacco leaf, entered the stem. Böning (7), as quoted by Henderson Smith, gives the following rates of movement in tobacco and tomato. In the former, at least two, and sometimes three or four, days were occupied in travelling a distance of 13 cm. in the basipetal direction, and acropetal movement within the leaf took place at rates of approximately the same order. In tomato the movement was slower within the leaf, *e.g.*, 9 cm. in three days for the mosaic virus. In the stem of tomato four days were necessary for traverse of 12 cm., four to five days for 20 cm. in the basipetal direction, three to four days for 12 cm., and four to five days for 25 cm. acropetally. Böning thought that the rate tended to increase as the virus progressed ; and considered that the rate was slower for the streak virus than for the virus of simple mosaic. In this connection some observations by the writer of the movement of two potato viruses X and Y in a mixture within the tobacco plant are relevant (49). When a young tobacco plant of the varieties White Burley or Virginia is inoculated by needle scratch with the virus complex X + Y, the first symptoms develop four days later under the writer's conditions (maximum temperature 80° F., minimum 50° F.). These initial symptoms take the form of double concentric rings, usually with a central spot, clustered on and around the needle scratches ; this is the virus X (see Fig. 1). Three days later, giving a total incubation period of seven days, the initial symptoms of the virus Y appear ; these take the invariable form of a

" clearing of the veins " of the youngest leaf (see Fig. 3). The next development is the appearance of necrotic lesions in the young leaves showing this clearing of the veins, indicating that the virus X has also reached the young leaves, but as the virus Y is already present there, the rings characteristic of X alone are not formed. Instead, a necrotic disease due to the combined action of the two viruses develops. It would appear, then, from a study of this symptom sequence that the virus X has the short incubation period of four days and appears first ; it is thus able to appear in its ring-like form upon the inoculated leaf as if it were a single entity. On the other hand, although virus Y takes a longer time to develop visible symptoms, it appears to move more rapidly through the plant and reaches the youngest leaves a day or two before virus X. There seems no doubt that these two viruses move at different rates through the young tobacco plant, and also they appear to be unequally distributed throughout the host, and this is particularly true in older, long-infected plants. In the case of tobacco plants long infected with the X + Y virus complex, it frequently happens that inoculation from the younger leaves yields only the Y virus, while the lower leaves still harbour both viruses.

Murphy and McKay (31) have studied the rate of movement of the leaf roll virus in the potato plant by infecting single-stemmed plants at the top, and then removing the lateral shoots at intervals and growing them as cuttings or, alternatively, by removing tubers at intervals. The leaf roll virus was proved to have reached all parts of the stem and tubers, from a scion grafted on at the top, after an interval of more than eight to ten days and less than to fifteen days.

In sugar beet it took six to ten days for infection with curly top to spread from the outer to the inner leaves, and when infection was made on the inner leaves the virus was not found in the outer leaves after six, eight or ten days (46). In small beet seedlings the times were shorter, *e.g.*, from inner to outer leaves in two days, from outer to inner leaves in four days. In large cucumber plants Doolittle obtained a higher rate, *i.e.*, 21 in. in twelve hours, or about 4·5 cm. per hour. As Henderson Smith has pointed out in his review (17), all these rates are very slow if the virus agent is carried in the transpiration current, and he quotes Sachs (43) as giving the rate of movement in the xylem of most potted plants at from 18 to 20 cm. per hour. These rates of movement of the various viruses are further proof, therefore, that the infective agent travels in the phloem and not in the xylem elements.

There seem to be only two instances recorded in which the rate of movement of the virus approximates to that considered normal for the xylem; these are the viruses of curly top of sugar beet (46) and streak of maize (55), and they showed maximum movement rates of 36 cm. per hour and 20 cm. per hour respectively. In both the above cases the experiments were carried out by means of insects, and Henderson Smith suggests that the virus may have been delivered directly into the conducting elements.

Grainger (16), working with the virus of tobacco mosaic, considers that the rate of spread of that agent in the leaves and stems of *Nicotiana tabacum* is a logarithmic one, beginning slowly and later accelerating.

In attempting to sum up the present state of knowledge of the movement of virus in the plant host, it will be well shortly to recapitulate the opinions of the different workers in this field. First as regards the possible methods by which the virus can move through its host; these are by diffusion, by means of the transpiration and translocation streams, *i.e.*, in the xylem and/or phloem vessels of the vascular bundles and finally by mechanical carriage in the streaming protoplasm. There seems to be good evidence that diffusion of the virus from cell to cell, possibly in the parenchymatous tissue, does take place, though the rate of movement of the virus in the plant is too rapid for this to be the sole explanation. Various workers, including Bennett, Holmes and Caldwell, consider that the agent moves in the phloem, but Grainger states that the movement takes place in detached leaves under conditions where transpiration and the processes of translocation were reduced to a minimum. As regards movement in the xylem, Bennett (5) and Caldwell (10) state that a virus is unable to pass a bridge of steamed tissue in the stem when the plant is inoculated above or below that bridge, thereby showing that the virus is not normally in the xylem. Caldwell (11) further considers that if virus is experimentally introduced into the xylem, it is unable to escape unless the cells are ruptured. On the other hand, Grainger (16), in precisely similar experiments, claims that the virus can move up or down across a bridge of steamed tissue in either stem or leaf. As Quanjer (38) and Bawden (4) have shown in studying certain " top necrosis " virus diseases in the potato, the disease starts with the development of necrosis in the phloem, so that the suggestion might reasonably be made that the virus is moving in that channel.

As regards mechanical carriage by the streaming of the protoplasm, this theory is not supported by the apparent differences in the rate of movement of the virus—slow near the point of inoculation and faster farther away. In conclusion, a statement of Grainger's may be quoted (16): " Must one look for the mechanism of spread in some function of the virus itself ? The observed rate could be given by a logarithmically increasing population free to move in an area of unlimited food supply and restricted as to direction."

Local Lesions

A method of iodine staining which renders local lesions conspicuous and reveals the points of infection has been developed (23). The technique is as follows : at suitable intervals after inoculation young plants are taken from the greenhouse during the afternoon. They are kept in a dark room at 10° C., usually from 5 p.m. to 9 a.m., and then transferred to a dark compartment at 22° C. The presence of virus causes abnormal starch retention in leaves of plants so treated. After six to eight hours at 22° C., leaves should be cut from the plants and immersed in 95 per cent. ethyl alcohol. They are allowed to remain in the alcohol overnight, or as much longer as is needed for the removal of their chlorophyll. The leaves are then stained in an aqueous solution of iodine in potassium iodide (60 g. KI and 20 g. I dissolved in 3 litres of water) for some hours, usually four or more, and finally rinsed in water to remove excess iodine and facilitate examination. Staining with iodine serves to detect starch in affected tissues (see Figs. 36, 37). The object of keeping plants at 10° C. for sixteen hours before storage at a higher temperature is to avoid too great loss of starch before morning, since young plants stored more than twelve hours at 22° C. in darkness generally lose almost all the starch from their infected leaves, even from areas affected by virus.

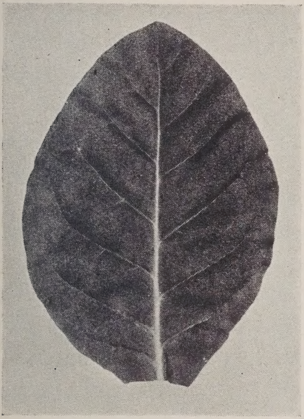


FIG. 36.—Tobacco leaf, three days after inoculation, by rubbing, with the virus of yellow tobacco mosaic, diluted 1:10,000. Very faint yellow-blotch local lesions are visible in places. (After Samuel.) (Compare Fig. 37.)

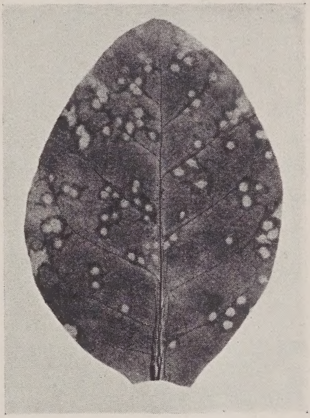


FIG. 37.—The same leaf as in Fig. 36, the same day, after killing, decolorizing in alcohol and treatment with iodine. The points of entry of the virus, which were visible on the fresh leaf (see Fig. 36) as very faint yellow-blotch local lesions or not visible at all, now show up very clearly, owing to the fact that no starch is formed at these points. Note the darker borders round the spots, where more starch is present than in the normal parts of the leaf. (After Samuel.)

A method suggested by Samuel (44) has been found useful for preserving stained leaves. The specimens are dried between sheets of waxed paper inserted between blotters in a botanical press.

Holmes' method not only constitutes a useful technique for the investigator of the local movement of the virus in its host but is also suitable for determining accurately the properties of a virus on a quantitative basis. If the accumulation of starch, which is picked out by this iodine technique, is due to the actual presence of virus and not only to the production of a lesion, then presumably Holmes' method would be applicable to other viruses than that of tobacco mosaic which do not produce local lesions on any host. Samuel (44) has applied these methods in studying inoculation with a number of different viruses, and he finds that temperature, age of the leaf inoculated and other factors influence the character and number of lesions formed. Price (35) has shown that certain varieties of garden beans (*Phaseolus vulgaris*) react with local lesions to rubbing with a virus of tobacco mosaic. Price has prepared curves indicating the possibility of using the number of lesions on susceptible bean varieties as a measure of virus concentration. Caldwell (12) has also applied the same method to studies with the virus of tomato aucuba mosaic upon *Nicotiana glutinosa*. He rubbed the filtered virus extract on the surface of detached leaves of that plant and kept them with their petioles in damp sand under a bell jar. First signs of spotting appeared within forty-eight hours.

Certain plant hosts react with certain plant viruses in such a way that the infective agent is localized on the inoculated leaf, and, spreading no farther, does not become systemic. *Nicotiana glutinosa* is a good example of this ; it gives local symptoms only with some tobacco mosaic viruses and the virus of tomato aucuba mosaic (see Fig. 38). On the other hand, it becomes systemically infected with, and usually killed by, the viruses of tomato spotted wilt and a mosaic virus from tomato studied by the writer (50). *Nicotiana glutinosa* is also systemically infected by the potato mosaic viruses X and Y, and by the virus of a vegetable marrow mosaic. Kunkel (28) differentiates tomato aucuba mosaic from ordinary tobacco mosaic by its capacity to produce local lesions in tomato and in certain species and varieties of *Nicotiana*.

Certain varieties of potato and *Datura stramonium* also give local infection only, with some tobacco mosaic viruses.

In studies upon the virus of tomato spotted wilt the writer has observed that the virus becomes localized (in the vast majority of cases) in the inoculated leaves of *Petunia* sp., and also, less frequently, in the inoculated leaves of *Solanum capsicastrum* and tobacco. In the case of tobacco it often happens that the virus spreads through the whole of the inoculated leaf which may become entirely necrotic, but for some reason the virus fails to reach the stem and the rest of the plant remains healthy. It is an interesting fact that the potato virus X very rarely becomes systemic in *Nicotiana tabacum* and allied plants if the latter are inoculated when they are more than a month or six weeks old. Well-defined local lesions develop, but usually no further infection of the plant takes place.

Holmes considers that there exists some interference with free and uniform movement of virus within the tissues of the leaf, and suggests that further study of this interference may help to explain the nature of those hosts which localize the virus in the inoculated leaf (23).

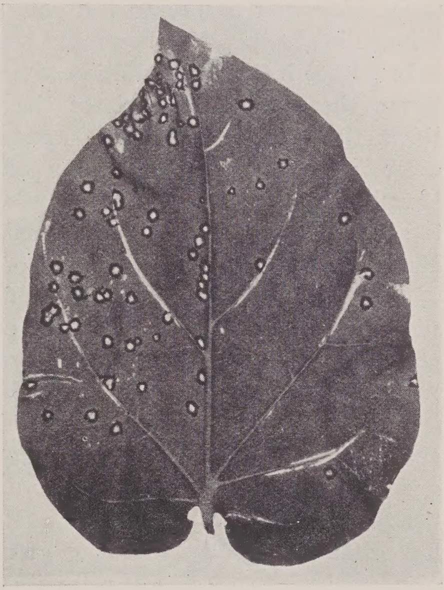


FIG. 38.—Primary or local lesions of tobacco mosaic on leaf of *Nicotiana glutinosa*, nine days after inoculation. This plant does not become systemically infected with the viruses of tobacco mosaic and aucuba mosaic of tomato. It does become systemically infected with potato virus X and the viruses of tomato spotted wilt and tomato stripe. (After Samuel and Bald.)

There are two cases recorded of local lesions produced by an insect vector—one by *Thrips tabaci* and the virus of spotted wilt on petunia (50), and the other by *Myzus persicæ* and the virus of cucumber mosaic on sugar beet (19).

Metabolism of Virus-affected Plants

In several virus diseases there occurs an accumulation of starch in the leaves of the affected plants. This is particularly marked in such diseases as potato leaf roll, " spike " disease of sandal, spinach blight, peach yellows and little peach.

In potato leaf roll in addition to accumulation of starch in the leaves there occurs necrosis of the phloem. Quanjer (37) considers that this accumulation of starch is due to the phloem necrosis which prevents transport of carbohydrates, but Murphy (30) and Bawden (4) consider that the phloem necrosis is secondary to the starch accumulation and leaf rolling, and Murphy has pointed out that rolling of the leaves and accumulation of starch occurs in the absence of phloem necrosis, which may be slight or absent in the first year of infection although starch accumulation and marked leaf roll are present. Murphy suggests that transport does not take place because the plastic materials are not present in a water-soluble condition. Thung (56), however, concludes that there is no transport in the diseased plants and that accumulation of starch is due to necrosis of the normal path of transport, *i.e.*, the phloem. Barton-Wright and M'Bain (3) have made a careful comparison of the carbohydrate metabolism of normal with that of leaf roll potatoes. They found by microchemical tests that starch begins to accumulate at a very early stage after infection, but examination of the phloem for necrosis at the same time was not made. Barton-Wright and M'Bain studied the variations in the carbohydrate content of laminæ and petioles of normal and diseased plants at hourly intervals over nineteen and twenty hours at different times in the growing season. Variations in radiation and temperature were also measured at the same time. The carbohydrates estimated in laminæ and petioles were reducing sugars (glucose and fructose), sucrose and starch. The results were calculated as a percentage of the residual dry weight. It was discovered that in healthy plants hexose and not sucrose was the first sugar of photosynthesis and, in healthy plants also, that sucrose was the sugar of translocation, and that there exists a high correlation between sucrose in the laminæ and sucrose in the petioles. In leaf roll plants, when the disease is in the "secondary" condition (see potato leaf roll, p. 298), it was found that photosynthesis was much reduced in the early part of the growing season, and the main reactions proceeding in the laminæ were conversion of starch to hexose, hexose to sucrose and sucrose back to starch, *i.e.*, the following reactions were in progress :—

$$\begin{tikzcd} & \text{Starch} \arrow[dl, shift right] \arrow[dr, shift left] & \\ \text{Hexose} \arrow[ur, shift right] \arrow[rr, shift left] & & \text{Sucrose} \arrow[ul, shift left] \arrow[ll, shift left] \end{tikzcd}$$

When the disease is in the "primary" condition, the same reactions as those described above are still in progress in the leaf, with the additional fact that photosynthesis is not reduced to the same extent as in secondary leaf roll.

In primary leaf roll, hexose in the laminæ is correlated with hexose in the petioles, and hexose is therefore the sugar of translocation in diseased plants. It was further discovered that hexose and starch were directly correlated in the petiole, and the argument is advanced that in leaf roll plants the hexose travels down the ground parenchyma and not down the phloem as in normal plants. In leaf roll plants sucrose is absent in the petioles at any time of the day or night and sucrose therefore plays no part in translocation in leaf roll material. Later in the growing season it was found that the starch content of leaf roll plants was much reduced and that the amount of photosynthesis had increased.

As regards the poor yield of tubers from leaf roll potato plants, this is usually considered to be due to necrosis of the phloem preventing free translocation to the tubers. Barton-Wright and M'Bain suggest an additional cause for this poorness of yield. Their analyses of the tubers show that in the healthy ones sucrose preponderates over hexose, but that the reverse is the case for leaf roll tubers in which the amount of sucrose is very small compared with hexose. It is therefore possible that not only is impaired translocation involved in the question of poor tuber yield, but that the nature of the sugars transported to the tubers must also be taken into account. It has been shown that sucrose is a highly efficient agent for the formation of starch, and it is possible that its absence may have something to do with the poorness in yield shown by leaf roll potatoes.

Studies on the photosynthesis of the sugar cane mosaic plant (13) seem to show that the normal plants and the green areas of diseased plants perform their photosynthesis in the normal manner, showing a large amount of starch in the afternoon and very little in the morning, while the white and light-green or yellowish areas showed a small amount of starch in the afternoon and practically none in the morning. It appeared as if the starch-forming power of the mosaic cane was reduced in proportion to the amount of infection while the power of translocation was almost unimpaired. This is the reverse of what happens in peach yellows, little peach and potato leaf roll, where the cells of the diseased leaves are filled with starch and translocation is greatly reduced or completely inhibited.

Freiberg (15) states that carbohydrates are more abundant in the dark-green than in the light-green areas, regardless of time of day. Proteins are present in both lighter and darker areas, but slightly in excess in the lighter areas.

According to Schweizer (45) chemical tests of the foliage from leaf roll potato plants showed that the leaves are characterized by more copious accumulation of sugar in the form of glucose than is found in the foliage of healthy plants. This abnormal sugar accumulation extends to the tubers that develop from diseased mother plants, but the effect is not noticeable until the period of winter storage. Chemical tests on dilute extracts from diseased mother tubers, after one to three months in the soil, showed that the proteins had all migrated to the shoots; this was not the case, however, with the reserve starch, the transport of which is evidently irregular. Microchemical tests made on sections through the stems of diseased plants with necrosis of the phloem revealed the absence in the phloem strands of the oxidizing body named leptomin by Raciborski, which has been regarded by other workers as a peroxidase.

Rouzinoff (42) states that the juice extracted from the leaves, stems, tubers and roots of leaf-rolled potato plants contains a well-marked excess of oxidizing ferments (oxidases and peroxidases) over the amount of these substances present in the juice of normal plants. *p*H values of the juice inside the stems and tubers indicate, according to Rouzinoff, that the juices are more acid in leaf roll than in normal plants. This last statement is not in agreement with the findings of Robertson and Smith (40), who state that the *p*H values of juice from healthy tubers, vars. Arran Comrade and Ally, were 5·64 and 5·80 as compared with 5·70 and 5·85 respectively in leaf roll tubers. Severe forms of mosaic such as crinkle, which may be considered as a mixed virus infection, gave rise to an increase in acidity.

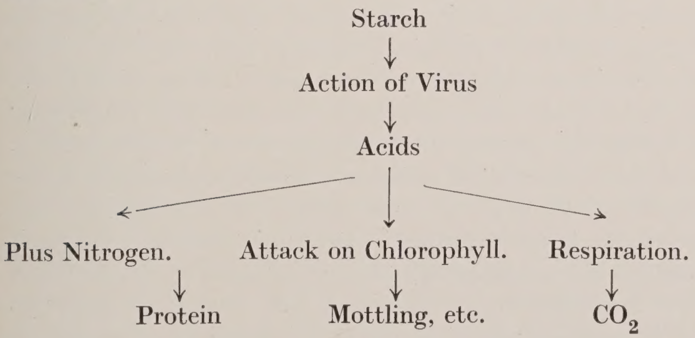
As regards the virus disease known as " spinach blight," (see p. 384), the starch content of the diseased tops is rather more than double that of the normal tissue. In the roots both total sugars and starch were practically alike in healthy and diseased plants. True and Hawkins (57) consider that the process of carbohydrate manufacture in spinach blight is not inhibited, though it may be retarded. In a study of the nitrogen constituents characteristic of spinach mosaic, Jodidi *et al.* (26) find that mosaic plants, particularly the tops, have a smaller percentage of total nitrate, acid amide, mono- and diamino-nitrogen, but a larger percentage of ammonia than normal plants, nitrous acid being present in diseased plants only. This is considered to be due to the fact that denitrification takes place whereby nitrates are reduced to nitrites which, reacting on the various nitrogenous compounds present in the spinach, bring about elimination of the nitrogen in the pure state, involving also loss of nitrogen in the form of ammonia. Very little denitrification, if any, takes place in the *roots* of diseased spinach. Similarly, in a mosaic disease of cabbage (25) denitrification takes place in the affected tissues, whereby the nitrates are in part reduced to ammonia which is lost as such, and in part to nitrites which, reacting on the amino groups of the various organic compounds, brings about elimination of elementary nitrogen. Denitrification occurs in a higher degree in the leaves than in the roots. This loss of nitrogen may in itself be an explanation of the chief disease symptom of cabbage mosaic, which is conspicuous dwarfing of the plant, owing to the fact that the nitrogenous compounds, such as acid amides, amino acids and others, which are partly lost through denitrification, are the very materials out of which the plant builds up its tissues.

Dunlap (14) has made a study of the total nitrogen and carbohydrates and the relative rates of respiration in a number of virus-infected plants. He investigated from these aspects, mosaic of tobacco, tomato, pokeweed (*Phytolacca decandra*), cucumber and raspberry ; yellows of peach, plums, asters and ragweed (*Ambrosia artemisifolia*) and leaf curl of raspberry. Results showed that the mosaic diseases were accompanied by an *increase* in the total nitrogen and a *decrease* in the total carbohydrate content of the foliage, while the reverse was true in the case of the yellows diseases. The carbohydrate to nitrogen ratio was decreased in the mosaic leaves and increased in those affected by yellows. The diseases of the latter group caused greater variations in the amounts of constituents in the leaves of their hosts than did the mosaics. There was an increase of respiration in young tissues infected with virus diseases, while in the older diseased leaves respiration was less than in healthy material.

It has been shown by comparative transpiration measurements on healthy and diseased potato plants of the Alma, Gisevius and Deodara varieties that wilting rolled leaves at first lose less water than healthy ones, but that sooner or later these values are reversed, the healthy foliage giving off less water than the diseased leaves. Comparative respiratory measurements indicate greater final intensity in healthy than in diseased leaves notwithstanding an initial increase in respiratory activity on the part of the latter. This phenomenon appears to be attributable rather to mechanical disturbances than to any lack of respiratory enzymes in the leaf roll plants (27). Thung (56) and Whitehead (58), however, state that respiration of leaf roll potato plants is greater than normal.

As regards virus diseases of the tomato plant, Rosa (41) states that leaves of tomatoes affected with " western yellow blight " (= curly top of sugar beet) show a decrease of total nitrogen while reducing sugars, sucrose and starch increase progressively with the external symptoms. On the other hand, Shapovalov (47), working with tomato yellows, which is apparently the same virus disease as "western yellow blight," finds an increase in the total nitrogen accompanied by a rapid accumulation of carbohydrates. According to Bolas and Bewley (6) a study of tomato plants suffering from aucuba or yellow mosaic has shown that, in the early stages of the disease, the removal of starch from the leaves is greatly accelerated (except at the point of infection) in plants placed in the dark. The local inhibition of starch removal at the points of infection is followed, often in about fourteen days, by the disappearance of starch over a larger area of the inoculated leaf, slight yellowing of the chlorophyll and failure of starch formation over the corresponding area in the light.

The acidity of an aqueous extract of infected leaves sampled at dawn, *i.e.*, immediately after loss of starch, is greater than that from healthy ones. A local absence of starch in the leaves even during the day appears to precede the development of mosaic symptoms. The freshly discoloured chlorophyll seems to react with copper salts, regenerating a green colour. At a later stage of infection a marked accumulation of starch is found in some parts of the affected leaves and complete absence in others. On the basis of these studies the authors suggest that the action of the virus on the starch may be represented by the following scheme :—



Read (39) has continued the investigations of Bolas and Bewley. Although sufficient results have not been obtained at present for any definite conclusions to be made regarding the effect of mosaic disease on the carbohydrate metabolism of the tomato plant, the preliminary results indicate that there is at first an increase over the controls in the amounts of both reducing and total sugars after inoculation with aucuba mosaic. This increase commenced about eight days after inoculation, and when at its maximum at about the twelfth day amounted to approximately 100 per cent. over the controls. Following this primary increase in the amount of sugars in plants inoculated with aucuba mosaic, there appeared to be a slight decrease in the sugar content commencing at about twenty days after inoculation. When the light intensity is so low that plants inoculated with aucuba mosaic gave distortion of the leaves without mottle or formation of yellow patches, it appears that the time during which the increase in sugar content persists is longer.

The transpiration rate in tomato plants affected with mosaic is higher than in healthy plants. There occurs a sharp drop in transpiration coincident with the appearance of the first visible mosaic symptoms, and this is followed by a gradual increase in the transpiration rate (18).

As regards the virus disease of sandal known as "spike," studies on the chemical composition of tissue fluids from the leaves and stem of infected sandal have been carried out in India by Sreenivasaya and Sastri (53, 54). It was found that a lower ash, a higher nitrogen, a lower calcium and higher maltose content and a higher content of reducing sugars characterized the tissue fluids of the spiked leaf. As to the chemical composition of the stem fluids, nitrogen and phosphorus concentration is higher as compared with the values for the healthy stems, but the calcium content in the diseased state is higher. A high gradient of calcium concentration exists from the stem towards the leaf which tends to flatten as the disease advances. It is suggested that the rapid transport of calcium through the stem is impaired by the incidence of spike; consequently, the leaf does not receive its adequate share of the calcium which, as shown by previous investigators, prevents abnormalities in the translocation of starch. Deficiency of calcium in affected leaves is a characteristic of spike disease, and it appears to be possible to diagnose the disease biochemically by determining the ratio of calcium to nitrogen. The sap of spiked sandal leaves seems to be more acid than normal, the *p*H of healthy sandal leaf-sap has been found to lie between 5·15 and 5·71, while in the case of spiked trees the figure runs between 4·69 and 4·99 (24). The total nitrogen in spiked leaves is generally greater than in healthy leaves, and the total nitrogen content varies with the locality. As physiological characteristics of the disease there may be put forward an increase in (1) total water-soluble nitrogen, (2) basic nitrogen and (3) total amino-nitrogen, and a decrease in the nitrate nitrogen in the diseased leaves, both in reference to the dry-weight of the leaves and in reference to the total nitrogen (52). Recently Sreenivasaya (51) has recorded the presence of mannitol in spiked leaves of sandal, which he considers to be one of the metabolic products of the virus. The diastatic activity of spiked leaves is much greater than that of healthy leaves, being sometimes four times as much (53). Similarly, the diastatic activity is greater in mosaic-diseased tobacco than in the normal plant (29).

Peterson (33) has studied the influence of three types of mosaic upon the plastid pigment and chlorophyllase contents of tobacco plants. The chlorophyll, carotin and xanthophyll contents were found to be reduced in tobacco leaves with mild dark green, light green and intense yellow mosaics. The greatest reduction of all three pigments resulted from the yellow type of disease, and the least from the mild dark green. The chlorophyll content and chlorophyllase activity of normal leaf tissue seemed to be directly correlated, whereas in mosaic plants the reverse was the case ; the pale green or yellowed tissues, though lower in chlorophyll content, were higher in chlorophyllase than the darker green leaf tissue.

The leaves of sugar beet plants affected with curly top have been found to have an oxidase content two to three times as great as the healthy and normally developed ones. No marked differences could be detected between the roots of the two kinds of plants, but an abnormally high oxidase content of the leaves was found in all plants, the growth of which had been retarded from any cause (9). Experiments carried out to ascertain the relative nicotine content of healthy and mosaic-diseased tobacco show that the nicotine content of diseased foliage is greatly in excess of that of healthy leaves, the difference in some cases being over 100 per cent. (48).

Metabolism of Viruses

Owing to the fact that no plant virus has yet been induced to multiply in a cell-free medium and to other technical difficulties, experiments have not been possible on the metabolism of a plant virus. Some work on this line, however, has been carried out with animal viruses in a few cases, and will be referred to briefly. Bronfenbrenner *et al* (8) have studied the question of independent metabolic activity on the part of a bacteriophage. Using a specially constructed microrespirometer, they were unable to detect CO_2 production by 10^{12} active units of bacteriophage during a period of ten days. Owing to the fact that the method does not reliably measure less than 0·005 c.c. of CO_2 there still remains the possibility that the agent does respire, but that during the experiment the amount of CO_2 produced was too small to be detected. More recently, using a different type of apparatus, Bachmann and Wohlfeil (2) also came to the conclusion that bacteriophage does not respire.

Holmes and Pirie (20) have recently made an attempt to estimate the growth of the filter-passing organism of bovine pleuro-pneumonia by estimating its metabolism. The infective agent was cultivated in meat broth, and it was found that there was little or no production of ammonia in cultures and no perceptible increase in amino-nitrogen (formol titration). There was a very slight fermentation of glucose during the first twenty-four hours of culture and a very large fermentation during the second twenty-four hours. This increase of metabolic activity is thought to indicate increase in the amount of organism present. The pleuro-pneumonia virus can reduce methylene blue in the presence of sodium lactate. The growth of the virus in culture can thus be conveniently estimated by following the increase in lactic dehydrogenase in the culture. No hydrogen donators other than lactate have been identified.

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CHAPTER X

SOME FURTHER ASPECTS OF THE STUDY OF PLANT VIRUSES

Recovery of infected plants—“ Carrying ” power—Resistance—Immunity—Attempted cultivation in vitro *of a plant virus—Some supposed causal organisms—Photography by ultra-violet light—Infra-red photography of virus symptoms in plants.*

Recovery of Infected Plants

Although it is exceptional for a plant affected with a virus disease to recover completely, authentic cases of such recovery are known, and they are briefly recorded herewith. It should perhaps be emphasized that by “ recovery ” is meant the disappearance of the *virus* from the tissues and not mere disappearance of the *symptoms*. This latter point is dealt with under “ carrying power ” and “ immunity.” Kirkpatrick (42), in further studies of leaf crinkle of cotton, states that he has observed at least one case of complete recovery from the disease. Bennett (9) reports recovery of Columbian raspberry plants affected with the alpha curl virus. Kunkel (45) finds recovery of certain varieties of sugar cane from mosaic a common occurrence, while Barber (2) has also recorded recovery from mosaic in the case of sugar cane of the thick tropical or “ noble ” type. Murphy and M’Kay (59) state that they have observed cases of recovery from crinkle and streak in potato Kerr’s Pink. Storey and McClean (81), in studying transmission of streak between maize, sugar cane and wild grasses, found that the maize virus frequently caused transitory infection of the Uba cane in the form of a few large chlorotic streaks on the leaves. All evidence indicated that the cane plant made a complete recovery from this transitory infection and ceased to harbour the virus. The writer has observed a similar transitory infection caused in tobacco by inoculation with a virus from lupins. In working with the virus of tomato spotted wilt upon the tobacco plant the writer has frequently observed the production of virus-free shoots (77) and also cases of complete recovery in the tobacco plant as a whole. These plants were all susceptible to reinfection with the spotted wilt virus. Price (66) has described the recovery of tobacco plants affected with a ringspot virus, but the recovered plants still retain the virus, and this point is further dealt with in this chapter under "immunity."

The following are references only to further cases of recovery from virus disease: Verwoerd (87) on two cases of recovery from a mosaic disease of tomato plants, and Brierley (16) on the same subject; Kunkel on grass mosaic in sugar cane (46); James Johnson (39) and Valleau and E. M. Johnson (86) record instances of recovery of potato plants affected with true tobacco mosaic. In working with a similar virus on potato, the writer has found that it does not become systemic in certain potato varieties (see also Blodgett (11)). In all the above cases—with the exception of that described by Price—the plants in question were liable to reinfection with the same virus.

"Carrying" Power

When a plant is systemically infected with a virus but is not visibly affected, it is spoken of as a virus "carrier." This phenomenon is not uncommon in the plant virus world and is of considerable economic importance, because such plants serve as unsuspected sources of infection to other more susceptible varieties. The best example of virus-carrying is to be found in the potato plant, some varieties of which habitually bear one or more concealed viruses. Certain varieties of hops carry the virus of hop mosaic without showing symptoms, and occasionally the same thing occurs with individual sugar beet plants and the virus of curly top. *Physalis alkekengi* has been shown by Nishimura (62) to carry the virus of tobacco mosaic. Some plants may give a slight initial reaction to infection with a virus and later carry it without symptoms. This occurs when *Solanum nigrum* (76) and the tomato plant (78) are infected with the potato virus known as " Y " (see Fig. 50). The subject of virus carriers in plants is one of great interest but, unfortunately, practically nothing is known of the causes underlying the phenomenon, or how the apparent balance between plant and virus is brought about. Holmes (38) has suggested the existence of some interference with free and uniform movement of virus within the tissues of an infected plant. He considers that further investigation of this interference may lead to a better understanding of the nature of certain symptomless carriers such as *Nicotiana glauca* R. Grah., in which there seems to exist a tendency to delay and minimize escape of virus to the tops of plants from inoculated, or subsequently invaded, leaves in which the virus may be multiplying rapidly. It should perhaps be made clear that a plant species which is a symptomless carrier of one mosaic virus is probably normally susceptible to, and will show symptoms of, infection by a second mosaic virus. Thus *N. glauca*, above referred to by Holmes as being a symptomless carrier of the tobacco mosaic virus, shows normal symptoms of systemic infection when inoculated with the virus of tomato spotted wilt. Similarly, various potato varieties which are symptomless carriers of one potato mosaic virus will show full symptoms of, and will even be killed by, a second potato mosaic virus. It is probable that in the case of the potato plant the " balance " of the carrier may be disturbed under certain circumstances. It does not necessarily follow that a potato variety will always remain a carrier of a specific virus, nor does it follow that a potato plant will carry a specific virus under all environmental conditions, such as in a glasshouse or out-of-doors, for example. The writer has some evidence to show that if a potato virus can be increased in virulence this fact may influence the carrying power of a potato variety. Thus, if the potato mosaic virus X, which is frequently carried without symptoms by certain potato varieties, be passed in progressive series through tobacco plants, its virulence appears to increase. If this more potent virus be now returned to a healthy potato plant of the " X-carrying " variety, it is found that the balance is disturbed and the more virulent virus is no longer carried without symptoms. It is possible, however, that this apparent increase in virulence of the X virus by progressive passage through tobacco plants can be explained on the theory of a selection during the progressive inoculations of the most virulent form of a number of strains of the X virus differing in virulence.

Salmon and Ware (74) have found that the addition of the virus of a chlorotic disease of the hop to hop plants which are already " carrying " a mosaic virus induces definite mosaic symptoms and causes the breakdown of the " carrying " capacity. It has been shown in the case of " spike " disease of sandal that the process of pruning or other mutilation of the branches will induce symptoms of " spike " at any time in an infected but apparently healthy tree.

Resistance

Some degree of resistance to a virus occurs commonly among individuals or varieties of plant species which are normally susceptible to that virus, and it is in the production of a virus-resistant variety where lies the best solution to the problem of virus diseases in economic crops. A few cases of varietal resistance to viruses are cited herewith : black raspberries (*Rubus occidentalis*) are more susceptible to mosaic than the red varieties (*R. idæus*) (69). According to Bennett (8) the following varieties of raspberries are very resistant to mosaic : Erskine Park, Sunbeam, Ohta and St. Regis. Jørstad (40) considers that there is some relationship between the varietal reaction of the raspberry to mosaic and the different meteorological and soil conditions. The variety Marlborough is considered to be particularly susceptible in acid soils and during seasons when the early summer is dry. Similarly a number of varieties of winter wheat are found to be resistant to the virus of wheat rosette (55).

Resistance has been widely studied in the case of sugar cane and its mosaic disease. Thus the varieties known as P.O.J. 213 and 228 are largely resistant to the mosaic virus, while the Javan varieties P.O.J. 2725, 2714 and 2878 appear to be resistant to streak. Some studies have been carried out in Louisiana on the varying degrees of resistance to mosaic shown by different sugar cane varieties (85). In old varieties such as D. 74 and Louisiana Purple, which show little or no tendency to recover from the disease, strains have gradually been selected that show the mosaic symptoms only in the mildest form. Of the four P.O.J. varieties used in the investigation P.O.J. 36 and 234 were found to be quite susceptible, while P.O.J. 213 and 228 may be classed as resistant. The relative resistance of these varieties is regarded as largely due to the capacity of the plants to throw off the disease and produce buds free of the virus. The comparatively resistant P.O.J. 213 and 228 produce a much greater number of healthy shoots from infected stalks than do the susceptible P.O.J. 36 and 234. The resistance shown to mosaic by the sugar canes D. 1135, Yellow Caledonia and H. 109 is considered to be largely mechanical and to be due to the layer of stone cells protecting the leaf from the punctures of the insect vector, *Aphis maidis* (47). Some varieties of a new sugar cane species have recently been described (13) and provisionally named *Saccharum robustum* Jesw. A marked characteristic of this variety appears to be freedom from mosaic coupled with vigorous growth. Brandes (13) states that in crossing *Saccharum spontaneum* (immune) with *S. officinarum* (susceptible) the seedlings produced in the F_1 generation were apparently immune, while a high proportion of similar material occurred in the F_2 generation. Susceptibility to mosaic would appear to be recessive in this particular cross, though the occurrence of the disease in C. 205, an F_1 hybrid of *S. spontaneum* and *S. officinarum*, has been reported in India and Florida.

Certain individual plants and selfed lines of the Chinese Long variety of cucumber are highly resistant to a mosaic virus known as "white pickle" (65). Occasionally susceptible individuals of the Chinese Long variety occur which have mosaic but with masked symptoms. Susceptibility to mosaic is dominant, but F_1 plants of Chinese Long × White Spine are less severely stunted than the susceptible parent. Seeds from a back cross of F_1 × White Spine produced plants yielding the short pickling type of fruit. Part of these are susceptible and part resistant, but no genetic ratios have as yet been determined.

A variety of spinach, known as "Virginia Savoy," is highly resistant to the virus of spinach blight (= American cucumber mosaic) (79).

In studies upon curly-top of sugar beet Carsner and Stahl (19) find that in every severely affected beet field a few individual plants stand out conspicuously less affected than the rest. Mackie and Esau (51), working on the susceptibility of beans to the virus of curly top, find that highly resistant varieties were found in the species of common beans and in the small Limas. Resistance to curly top appears to be partially correlated with pink and red colours. Combination of resistance to both curly top and mosaic is possible in a single variety of bean. As regards the resistance of different varieties of tomato to this virus, this has been found to be weak, and when present appears to be due rather to a tendency to escape infection than to a tolerance of the virus (49).

As regards potato virus diseases, a recent statement in Science (41) reports the production of a new potato variety, known as Katahdin, which is thought to be resistant to the virus of " mild mosaic." Through the courtesy of Mr. Stephenson, the writer has received a small supply of Katahdin for experimental purposes and it may be of interest to record some preliminary inoculation experiments with this potato. The potato viruses used in the experiments are those known as X and Y (78) and they were inoculated to the plants by rubbing and by needle scratch. It was found that both viruses gave visible local symptoms on the inoculated leaves ; round necrotic lesions in the case of X, and streak-like lesions on the veins in the case of Y. Further development of the X infection took the form of a mild mosaic mottling on the topmost leaves, while the other leaves developed a curious network of faint necrotic lines most pronounced on the undersurface of the leaves and unlike the reaction of other potato varieties to this virus.

The behaviour of the Y virus on Katahdin was interesting in that symptoms of systemic invasion did not develop in the year of infection. Plants arising the following year, however, from the tubers of these plants developed the symptom picture typical of current season infection with the Y virus, viz. : severe necrosis of the leaves and leaf drop streak (see Fig. 50). It seems clear, therefore, that the potato Katahdin is not resistant to the viruses X and Y.

The resistance to virus infection exhibited by certain plants and plant varieties is often more apparent than real, and may consist merely in the fact that for some reason, possibly purely mechanical, the plant is avoided by the insect vector and so escapes infection. This is probably the case with some potato varieties. Similarly, the resistance to mosaic shown by certain varieties of sugar cane is thought to consist of a capacity on the part of those plants to recover rapidly from the disease and produce buds free of virus (85).

Immunity

On the whole it is probably true to say that plants do not develop an immunity to virus diseases, and this fact is made clear in the section dealing with recovery where, with one exception, all the recovered plants were again liable to infection. East (27) records the fact that in sugar cane there may be as many as three successive infections with, and recovery from, mosaic, recovery occurring at any time between three and twenty-four months after infection. The results of tests, while far from conclusive, give some indication of a consistent difference in precipitin reaction between Cristalina-type canes known to be free of mosaic virus and those which have or may have had the disease.

According to Thung (84) when the virus of common tobacco mosaic (= Johnson's tobacco virus 1) and of white mosaic (= Johnson's virus 6) are mixed in equal parts and inoculated, the inoculated plants develop the symptoms of both diseases and the infective principles of each are recoverable. If, however, a tobacco plant infected with white mosaic is inoculated with the virus of common mosaic, or *vice versâ*, the symptoms of the second-inoculated virus fail to develop. In other words, the presence of one of the two viruses protects against infection by the other. Thung considers that a certain antagonistic action must be admitted to exist between a cell in which one of the two viruses is completely reproduced and the second virus.

Salaman (73) claims that a similar state of affairs exists with regard to virulent and weak strains of potato virus X. He finds that tobacco or *Datura* plants, already infected with the weak strain of X, give no reaction when inoculated with a virulent strain of the X virus.

A recent paper by Price (66) describes an apparent acquired immunity to a ringspot virus in *Nicotiana.* Price has found that various species of *Nicotiana* when inoculated with the ringspot virus develop a typical and severe disease. Later the plants gradually recover and finally appear normal. Such recovered plants, however, invariably contain the virus equally as virulent as that contained in plants still showing symptoms of the disease. This recovery appears to take place under all environmental conditions and cannot be compared with the masking of symptoms in certain virus-affected plants, such as potatoes, which occurs under exceptional environmental conditions. Further inoculation of such recovered plants with the ringspot virus fails, as would perhaps be expected, to induce fresh symptoms of ringspot. Here then is the production of an acquired immunity of a kind, although it is not perhaps an immunity in the usual conception of that term. It is not certain whether the ringspot virus actually continues to multiply within the recovered plants, although the fact that plants produced from cuttings of recovered plants still contain the virus would seem to suggest that multiplication of the virus does take place. Something of the same phenomenon is shown by inoculation to tomato with the potato virus known as Y (78). The tomato plants give an initial reaction and develop faint symptoms which rapidly disappear. The plant then carries the virus without further symptoms.

As Price has pointed out, certain animals after recovering from a virus affection develop an immunity which appears to be associated with the presence of active virus. There is some evidence that active virus may persist in certain cases within the recovered animal. Thus Cole and Kuttner (20) showed that old animals were carriers of the salivary gland virus of guinea pigs, but that the virus was not obtainable from guinea pigs less than one month old. They found that this virus could be obtained at any time from a pig once infected, and that it was highly infectious for guinea pigs not previously infected. Similarly, de Kock (43) found that the virus of infectious anæmia was present in the blood of horses as long as seven years after an attack of this disease. It seems possible in this latter case, however, for a horse to suffer a relapse or " reinfection " with this virus. Farber and Wolbach (29) describe the occurrence of inclusions in the submaxillary glands of infants mostly under one year of age. These inclusions are apparently identical with those found in the submaxillary glands of guinea pigs, and are generally similar to inclusions which are found in diseases due to filterable viruses and lead to the suspicion of the existence of a disease in infants of filterable virus ætiology. However, if that be true, there were no distinctive chemical or pathological features which would permit its recognition. Again, Covell (21) describes the occurrence of intranuclear inclusions in monkeys, unaccompanied by specific signs of disease, and says that it seems likely that monkeys must now be listed with humans, guinea pigs, rats, rabbits and dogs as animals in which one or more viruses sometimes lurk, capable of producing intranuclear inclusions in the absence of recognizable clinical symptoms.

While it is probably true that, with the exception of the case described by Price, there is no *acquired* immunity to viruses in plants, certain species of plants do appear to have a natural immunity to a specific virus which affects other closely allied members of the same group. Thus, for example, the potato mosaic virus known as Y seems unable to infect the Solanaceous plant *Datura stramonium* (78), although this plant is very susceptible to another potato mosaic virus, X.

Holmes (38), discussing the natural immunity in Solanaceous plants to the virus of tobacco mosaic, considers that there exist tendencies of the three following kinds towards immunity : (1) a tendency opposing infection at time of inoculation ; (2) a tendency opposing increase of virus after infection ; (3) a tendency opposing spread of virus. These might be spoken of briefly as partial resistance (*a*) to infection by virus, (*b*) to increase of virus and (*c*) to spread of virus. Holmes describes an interesting condition in the genus *Physalis*. One species, *P. angulata*, produces a systemic necrosis with tobacco mosaic virus; another, *P. alkekengi*, may act as a symptomless carrier of the virus; *P. peruviana* becomes systemically infected and displays obscure mottling and defoliation, but *P. viscosa* has given no indication of susceptibility to infection thus far.

While it is generally recognised that antibody production and related phenomena, as they are associated with the animal body, play no direct part in the immunity of the plant, Leeman (48) has shown that certain extracts and organic substances play a considerable part in stimulating the resistance of a plant to attack by fungus disease. The substances, when added to the soil, are harmless to the parasite, but become detrimental when taken into a plant and incorporated with its protoplasm.

Attempts have been made to induce immunity in plants by inoculating them with virulent or attenuated cultures and also by injection with products of bacterial metabolism. As early as 1903, Hiltner and Störmer (34) reported that legumes infected with a virulent root-nodule organism could not be reinfected with a less virulent one. A similar conclusion was reached by Dunham and Baldwin (22). A number of investigators have reported successful or partially successful immunization of plants against various pathogenic organisms by exposing them to attenuated cultures or to extracts of pathogens. This work has been summarized recently by Carbone and Arnaudi (17). Although the evidence reported by these writers indicates that the treated plants were more resistant to attack than were untreated controls, it is not clear from this evidence whether the increased resistance was due to a specific reaction against the pathogen or to the poor growth made by the treated plants (66). Carbone and Kalajev (18), in continuing their studies on the artificial immunization of beans (*Phaseolus vulgaris*), against *Botrytis cinerea*, reply to this criticism by showing that the immunizing capacity of the fungus extract varies according to the mode of preparation and does not strictly correspond to their toxicity. They consider that this invalidates the suggestion that the intensified resistance of the inoculated plants is only an accompaniment of recovery from the disease.

In this connection it was further shown that plants grown under adverse conditions and also deliberately maltreated were not more resistant than the controls to infection by *B. cinerea*, but on the contrary more susceptible. In all the plants in both series of tests the fungus develops in the early stages, and only in the pre-inoculated individuals does the mycelium subsequently disappear and the wound heal. Carbone and Kalajev consider that these data support the assumption that vaccinal immunity in plants is a vital specific phenomenon.

Some attempts have been made by Silberschmidt to demonstrate the presence of antibodies in plants (75). It was found impossible to induce passive immunization of healthy tobacco plants by inoculation with a mixture of tobacco mosaic virus and anti-virus serum (obtained from inoculated animals). Negative results were also obtained in attempts at the active immunization of tobacco plants by inoculation with dilute suspensions of the virus.

It does not lie within the scope of this book to do more than touch upon the question of acquired immunity in animals to virus disease. As already pointed out, a characteristic of many virus diseases of animals is the lasting immunity which they confer. Andrewes (1), discussing this question, gives the following list of animal virus diseases which confer a lasting immunity: smallpox, measles, mumps, chicken pox, herpes zoster, poliomyelitis, yellow fever, cattle plague, swine fever and dog distemper. The following viruses confer a transient immunity only: common cold, herpes simplex, foot-and-mouth disease and influenza. As regards the persistence of a virus in an immunized host, in addition to the two cases given above, viz: the virus in the submaxillary glands of guinea pigs and that of infectious anæmia of horses, Andrewes states that an organism like *Bacillus coli* may permanently " carry " a bacteriophage which does not affect it but can cause lysis of another organism, the dysentery bacillus.

It will be convenient to consider here some immunologic reactions of the tobacco mosaic virus. Purdy (68) has experimented on the reactions of the rabbit to inoculation with juice from mosaic tobacco plants and appears to have obtained some fairly definite results. Separate antisera were produced in rabbits to normal sap from healthy Turkish tobacco plants and to virus sap from mosaic ones. The immunologic reactions of the antisera were studied by means of alexin-fixation tests. Precipitin-absorption tests were made with the same tobacco virus-multiplied in tobacco, tomato, pepper (*Capsicum annuum*) and petunia plants,—and experiments were carried out on the inactivation properties of both antisera and normal serum on virus sap. The results of Purdy's experiments showed that the normal and virus saps of tobacco possess common antigenic substances. The normal and virus saps of tomato, pepper and petunia plants contain antigenic substances in common with the virus sap of tobacco which are either absent or present only in small quantities in normal tobacco sap. The two antisera possess alexin-fixing antibodies and precipitins in common. All the precipitins to normal tobacco sap may be removed from either antiserum by absorption with the virus sap of tobacco. Specific precipitins for virus sap of tobacco, tomato, pepper and petunia are present in the antiserum to tobacco virus sap and cannot be removed by complete absorption with normal tobacco sap. Appropriate quantities of the antiserum to the virus sap of tobacco are capable of completely inactivating the virus sap. This preventive action is not manifested by corresponding quantities of antiserum to normal tobacco sap or normal rabbit or guinea pig serum. There is some evidence that a lytic specific antibody to virus sap is present in the homologous antiserum. In a later paper Beale (= Helen Purdy (6)) states that no specific precipitate for virus extracts of tobacco affected by ringspot or cucumber mosaic was demonstrable. Beale considers that the specific antigenic substance in the virus extract of tobacco mosaic is foreign antigenic material, possibly virus itself, rather than altered host protein. Matsumoto (53), working on the antigenic properties of tobacco mosaic juice, has studied the serological behaviour by means of precipitin reactions. The antigens used for the test consisted (1) of ultra-filtered mosaic juice and (2) of centrifugalized supernatant liquid of the expressed mosaic juice without ultra-filtration. To each of these solutions was added an equal part of the antiserum at varied dilutions. Results of readings indicated that precipitations occurred in the antiserum-virus mixtures of the two different preparations. No specific precipitation was observed when either the normal serum or the normal tobacco juice was used. Tobacco plants were inoculated with the supernatant liquid and the precipitate of the antiserum-mosaic juice mixture. It was found that the infective principle of the virus had disappeared in every case from the supernatant liquids of the antiserum-virus mixtures, whence it may be inferred that the infective principle is precipitated by a specific action of the antiserum. Normal rabbit serum was found to be definitely incapable of destroying the infective quality of the virus which was detected practically unimpaired in the supernatant liquid of the normal serum-virus mixtures as well as in the precipitate of the same kept in contact for forty-eight hours. Mulvania, however, states (58) that he was unable to recover the virus of tobacco mosaic from the normal blood of a living rabbit. Matsumoto and Somazawa (54) have carried out studies to determine whether the antigens of tobacco mosaic virus, which can stimulate the production of specific precipitating antibodies when injected into a rabbit, occur in the parts of infected plants showing no recognizable mosaic symptoms and how they are formed and spread. All parts of the diseased plants were found to contain the specific antigen, even when there were no symptoms. Root extracts were also found to be capable of producing specific antibodies when injected into rabbits. The concentration of the antigenic substance was measured in the different parts of infected plants. Antigenic activity was found to be highest in dried leaves and about equal in the expressed juices of fresh leaves and of fresh and dried roots. In stems, especially dried ones, the antigenic concentration was very low, notwithstanding the fact that heat desiccation produced no injurious effect on antigenic activity. Tests carried out to ascertain whether the formation of the antigen takes place parallel with the multiplication of the infective agent showed that no noticeable reaction followed the use of juices prepared from portions of inoculated plants until four or more days after inoculation. The antigen was distinctly recognizable in the apical and subterranean portions of the plants before mosaic symptoms appeared. In a series of tests to determine the distribution of the antigen in various parts of the plants, it was found to appear in the portions above the point of inoculation four days after the operation, the underground parts developing it later. It is inferred that the antigenic reaction is actually due to the infective agent and not to modifications of the host protein.

Beale (7) has attempted to use the serologic reaction as a means of determining the concentration of tobacco mosaic virus. Green-leaf extracts of Turkish tobacco affected with mosaic (Johnson's No. 1 virus) were tested for their ability to form precipitates when mixed with serum of rabbits, hyperimmunized against homologous extracts. The concentration of active virus in each extract was then determined independently by the number of local lesions resulting from inoculation of *Nicotiana glutinosa*, according to the method of Holmes (36). Beale states that a correlation was found to exist between the virus concentration of the various extracts and their ability to precipitate antisera. It is suggested that the precipitin reaction may be of value for rapid and accurate determination of the relative concentration of virus extracts.

Attempted Cultivation in vitro of a Plant Virus

In 1924 Olitsky (63) considered that he had demonstrated the multiplication *in vitro*—*i.e.*, in a cell-free medium—of the virus of a tomato mosaic. Using no unusual or special technique, Olitsky inoculated sterile normal tomato juice with the juice of mosaic tomato, sub-cultured from this into normal juice, and obtained infection even with the twelfth sub-culture. This represents a dilution of $4/10^{16}$, far outside any possibly infective dilution of the original inoculum. Since that time a number of plant virus workers have attempted to repeat this work of Olitsky, notably Mulvania (57), Goldsworthy (29A), Purdy (67), Grainger (31) and Henderson Smith (32) using tomato and tobacco mosaic viruses, and Goss (30) using the virus of potato spindle tuber. All these attempts to cultivate the viruses in a cell-free medium failed. One such negative attempt to cultivate the virus of aucuba or yellow mosaic of tomato, as carried out by Henderson Smith (32), is described here in order to illustrate the methods used in this type of work : " Sterile tomato juice was obtained from young, actively growing, normal tomato plants by the same method as was used in the preparation of filtered virus juice, the crude juice in this case being passed through filter-paper, instead of sand and paper-pulp, before being passed through the L_1 and L_3 candles. After final filtration it was distributed in 5 c.c. volumes and incubated for one week at 27° C. Successive batches of such juice were prepared from time to time, and no juice was used that was more than three weeks old. The pH of this normal juice in four successive batches was 5·5, 5·1, 5·5 and 5·5, tested colorimetrically. After incubation, tubes were inoculated in the following manner from young tomato plants showing recent and well-marked signs of the aucuba disease. Petioles, or young stems, were cut across with a sterile scalpel, seared at the end with a red-hot knife, capillary tubes inserted through the seared surface and small quantities of juice sucked up and transferred to tubes of the medium. In all, 21 tubes were so inoculated, constituting the first sub-culture, and were then incubated at 27° C. After five days, from each of these tubes 0·1 c.c. was transferred to a tube of fresh medium (a separate pipette being used for each tube) ; this constituted the second sub-culture. The process was repeated every five days, giving the five-day series of cultures. Similarly, every ten, fifteen and twenty-five days sub-cultures were made, giving the ten-day, etc., series. There were, therefore, four series of sub-cultures, differing in the periods of incubation. Any tube showing obvious contamination was rejected, but this rarely occured. From time to time tests of the sub-cultures were made by inoculation to plants. For this purpose, from each of all the tubes of a sub-culture in any one series 0·2 c.c. was withdrawn as a sample, the samples mixed together, and the mixture inoculated to young tomato plants in active growth. Inoculation was made by needle prick, at least eighty punctures per plant in four leaves, and, further, a small pledget of cotton wool soaked in the juice was inserted into an incision in the stem. The plants were held for at least five weeks, and examined regularly. The batches of uninoculated medium were tested in the same way, always on six to eight plants.

" The results of all inoculations made up to the fourth sub-culture are shown in the table. The amount of the original inoculum of infected material could not be exactly measured, and varied a little in every tube with the quantity of juice taken up in the capillary, which also took up small pieces of tissue. It was estimated to be about 0·01, and not to exceed 0·05 c.c. in any tube. Taking the larger figure as an outside estimate, the dilution of the inoculum was in the first, second, third and fourth sub-cultures, 1×10^2, 5×10^3, 25×10^4 and 125×10^5 respectively. The third sub-culture, then, represents a dilution of the original inoculum of 1 in 250,000, a dilution still possibly infective; but the fourth sub-culture, being a dilution of 1 in 12,000,000, is outside the range of still infective dilution. As is shown in the table, in no case was infection got with the fourth sub-culture (nor in several examinations of later sub-cultures); but in one at least of the series the third sub-culture was still slightly infective. No evidence, therefore, was obtained of multiplication of the virus.

TABLE

Series (days).	Sub-culture.	No. of Plants.	No. Positive.	% Positive.
5	1st	4	2	50
	2nd	6	1	16·6
	3rd	8	1	12·5
	4th	10	0	0
10	3rd	6	2	33·3*
	4th	8	0	0
15	4th	8	0	0
25	4th	8	0	0

* This figure is unreliable, since the test of the batch of medium used for this sub-culture gave one positive result.

" This experiment was repeated the next season, using as normal medium a juice differently prepared. Here the tissue was ground up in an apparatus devised by W. A. Roach (72), and so finely that no intact cells could be detected under the microscope; the liquid was then passed through an L_1 and then an L_3 candle. The preceding experiment was then repeated, using only one series, viz., seven days' incubation, and the original inoculum consisting of 0·1 c.c. of filtered virus juice, which was proved to be very active. Eight plants were inoculated with each sub-culture: the first sub-culture gave seven positive, the second two, the third, fourth and fifth none."

The method used by Goss (30) in attempting to cultivate the virus of potato spindle tuber is briefly as follows: cores of healthy tubers were removed aseptically and immersed in 10 c.c. of sterile water in test tubes. Similar cores of spindle-tuber potatoes were used in other tubes. After incubation for two days at 25° C., 1 c.c. of the liquid in a tube of spindle tuber tissue was transferred to a tube of healthy tissue. Transfers were subsequently made every twenty-four hours to new tubes of healthy tissue. After each transfer the core of tissue from the tube inoculated the previous day was used to plug into the cavities of healthy seed pieces. The cultures made in this way resulted in a series of dilutions ranging from 1 : 10 up to $1 : 10^{11}$. The test was run in triplicate. None of the plants grown from the inoculated seed pieces developed the disease.

In 1931 Olitsky and Forsbeck (64) made a fresh investigation of Olitsky's original work, and their conclusion is given here: "In nearly every case, irrespective of the method employed, there was some increase in the potency of the virus beyond the original titration, as was also observed by McKinney. In the earlier experiments this increase was much greater than in the later ones, possibly on account of some cyclic variation in the virus. The question arises whether the increase was due to an activation or a dispersion of aggregated virus particles by some unknown mechanism, or whether it represented some actual multiplication of a living agent. The latter interpretation cannot be definitely accepted on the basis of the present data." It can therefore be stated that up to the present no plant virus has been cultivated *in vitro*.

It may be instructive briefly to consider here some parallel experiments in the cultivation in lifeless or cell-free media of certain animal viruses. The cultivation of animal viruses in the presence of *tissues* surviving *in vitro* such as has been demonstrated by Maitland and Laing (52) and others is not touched upon here. Eagles and McClean (26) have recently reported that vaccine virus is capable of regeneration in a cell-free medium. Owing, however, to the possibility that the medium employed was not completely cell-free and the multiplication of the vaccinia virus may not, therefore, have taken place in the nutrient material in the absence of cells, the work was repeated under conditions where no whole cells could be demonstrated. Eagles and Kordi (25) state that vaccinia virus has been cultivated through ten generations in sub-culture in a medium of ground kidney emulsion in which no whole cells could be seen. A total multiplication of 10^{20} was obtained. It is suggested that substances are released from the cells during the preparation of the medium which are, in the fresh state, essential to growth of virus. The virus did not appear to be altered in any way by this cultivation *in vitro* as evidenced by inoculation tests, etc. Large numbers of Paschen bodies were found to occur in the cultures. On the other hand, attempts by other workers, notably in the United States, have failed to demonstrate the multiplication of vaccine virus in cell-free media (see Li and Rivers (50): Muckenfuss (56A): Rivers, Haagen and Muckenfuss (71)). The only apparent virus agents which are generally accepted as cultivable in lifeless media are those of pleuro-pneumonia of cattle and of agalactia of goats, which readily multiply in broth. There are, however, some virus workers who do not place the causative agent of bovine pleuro-pneumonia in the virus group, but consider it rather as allied to some obscure group of fungi. It may be impossible, as Rivers has pointed out (70), to cultivate many of the viruses as they may be obligate parasites (like the malarial organism), incapable of multiplication in the absence of living susceptible cells.

Eagles (23, 24) has recently reviewed the subject of the *in vitro* cultivation of animal viruses and the reader is referred to his two papers for further information.

Some Supposed Causal Organisms

From time to time there appear in the literature on plant viruses descriptions of organisms of various types which are considered to be the causative agents of plant virus diseases. Leaving on one side the question of intracellular inclusions, or X-bodies (see Chapter II, p. 26) which are sometimes looked upon as living agents of disease, the other " causative organisms " may be grouped under the headings of protozoa, bacteria or fungi. Some work by Ray Nelson (60) attributed the virus disease of bean mosaic to a protozoan, while Eckerson (28) gives a description of certain organisms observed in tomato mosaic. Twenty-four hours after the inoculation of alternate leaflets of a series of young tomato plants with filtered juice from mosaic plants, minute flagellate organisms were observed in the veins and adjacent mesophyll cells of the leaflets opposite to the inoculated ones.

Several authors have associated bacteria with virus diseases. Ray Nelson (61) describes a small coccus in the seeds of mosaic-infected beans. He finds these cocci regularly present in the seeds of infected plants and considers that the results of his bacteriological studies in conjunction with the results of the seed-transmission tests indicate that in these diseased plants there is a close association of the coccus and the virus, whatever the latter may be. However, inoculations of the cocci isolated from plants affected with true mosaic disease and of a pleomorphic organism isolated from plants affected with rugose mosaic have not resulted in the reproduction of mosaic disease in the inoculated bean plants.

In a recent letter to *Nature*, Bewley (10) considers that he has evidence connecting a tomato mosaic virus with a bacteriophage and puts forward the suggestion that the mosaic virus is a bacteriophage which has become adapted to a free life within the tomato plant. Swezy and Severin (82) report the discovery of rickettsia-like micro-organisms associated with curly top of sugar beet. They state that there are two types of these micro-organisms which cannot be separated on morphological grounds. The difference between these two apparent organisms lies in the fact that one may be filtered through a fine Berkfeld filter while the other is unable to pass through such a filter ; the one being found only in infective insect vectors of the virus and the other occurring in both healthy and infective insects. There appears, however, to have been no additional or confirmatory evidence of the above claims since their publication in 1930.

Takahashi and Rawlins (83) have examined a stream of clarified sap from mosaic-affected tobacco plants by means of polarized light and, basing their findings on the stream double refraction, they consider that they have evidence of the existence of rod-shaped bodies in the virus-containing sap. Other writers who have associated bacteria with the causative agent of plant virus diseases are Melhus (56), Boncquet (12) and Smith and Boncquet (80). v. Brĕhmer and Bärner (15) and v. Brĕhmer (14) state that they have discovered the causal organism of potato mosaic, possibly potato leaf roll and of beet mosaic. They describe yellowish green, oval, amorphous bodies which they consider are organisms belonging to a hitherto unknown systematic group of the Archimycetes, allied to the Plasmodiophoraceæ. In his paper, v. Brĕhmer (14) states that the causative organism of potato mosaic and possibly of potato leaf roll is a myxomycete, *Plasmodiophora solani,* while that of beet mosaic is *P. solani var. betæ.*

In most of the above cases it must of course be assumed that the various " causative organisms " are capable of existing in a filterable stage.

In considering the relationship of the varied organisms above described and the plant viruses with which they are said to be associated, it is clear that much more research is needed before it can be said that any close connection between them has been demonstrated. Indeed, some recent work by Henderson Smith (33) seems to disprove any connection between plant viruses and visible bacteria. He was able to show that from the necrotic tissues of tomato plants suffering from streak (= stripe, see p. 373), a virus disease, it was possible regularly to obtain bacteria which belong to one or both of two definite types. The same bacteria occur in tobacco plants infected with the same disease. If the plants are grown from sterile seed under aseptic conditions throughout and inoculated with bacteriologically sterile virus-holding juice, those bacteria do not appear although the plants develop well-marked signs of the disease. Henderson Smith's conclusion is that there is no evidence that the bacteria are derived from the virus or the virus from the bacteria.

Photography by Ultra-violet Light

In the literature on plant viruses there appears to be only one record of an attempt to photograph the virus particle by means of ultra-violet light. This attempt was made by Holmes (35) using the ultra-violet light photomicrographic apparatus devised by Köhler (44). The apparatus is constructed to allow the sorting out of light of low wavelength from the cadmium spark, and the isolation of a relatively pure beam of light corresponding to the wavelength of 275 $\mu\mu$. This light is led through a series of quartz prisms and passed through the microscope, which is provided with quartz lenses adjusted for this exact wavelength. Since the light is monochromatic, no chromatic aberration interferes with the sharpness of images secured. Objects to be photographed must be unstained and mounted between quartz slides and cover slips to ensure complete transmission of the ultra-violet light. The virus-containing juices used were quite satisfactory, in that they contained just enough debris to make it certain that focussing on the several layers of a given mount was accurate, and that suitable small objects within the range of the instrument were being depicted. The viruses used by Holmes were chosen to represent as varied types as possible. They were the following : aster yellows, tobacco mosaic, tobacco ringspot, potato witch's broom, potato leaf roll, potato rugose mosaic and potato aucuba mosaic. The juices to be photographed were obtained from the petioles of leaves of affected plants by pressing these against the quartz slide to secure a drop of fluid together with some debris from the broken cells. The fluid was mounted for photographing between a quartz slide and a small quartz cover slip, and sealed in place with a ring of vaseline. After a rough focus had been obtained by means of an air bubble purposely admitted into the preparation, a more careful adjustment of the focus by means of the fluorescent finder was made. A series of strip pictures was then taken with a low-power quartz ocular, the fine adjustment setting being recorded at each exposure. The plate thus secured was developed at once. With the best focus established, the low-power ocular was replaced by a higher, the lenses being well enough made to be in absolute agreement in giving a sharp focus. The same quartz objective was used throughout. In his investigation, Holmes was concerned only with the range between the limit of resolution of the visual microscope, with particles in the neighbourhood of 150 $\mu\mu$ in diameter, and the limit of the ultra-violet light instrument with oblique light with particles approximately 75–80 $\mu\mu$ in diameter.

The results obtained from Holmes' study were negative. In the virus-containing juices of the seven diseases investigated, no formed particles were observed which differed from those depicted in the juices from corresponding healthy plants.

Barnard, by means of his ultra-microscope and using ultra-violet light has photographed the virus particles of certain animal viruses, notably of infectious ectromelia of mice, and he describes his microscopical methods as follows (4). Fresh material containing the virus in living condition was used without fixation or staining. The following paragraphs are quoted from Barnard's description.

" Two separate but related methods were employed. In one the granular elements in a preparation were observed by means of a high-power dark-ground illuminator, the objective having the highest numerical aperture employable when the object is immersed in a watery medium. This limit is reached with an objective of 1·27 N.A., but one of 1·22 N.A. can also be used. As a light source a quartz mercury vapour lamp provides a luminous area of uniform intensity and remains constant for long periods. Two microscopes are used, both are illuminated from the same source and are provided with identical optical systems, to ensure exactly comparable images. Infective material can thus be examined side by side with any desired control. Monochromatic green light of wavelength 5461 Å.U. is obtained by a suitable colour screen and provides an illuminant whereby slight differences in intensity can easily be appreciated. The identification of bodies of a constant order of size or of luminosity is therefore comparatively easy, and with practice considerable accuracy is attainable. This method has been applied to membrane filtrates and concentrates with the result that bodies can be identified by visual means although the image gives no real indication of size or form. This process is essentially preliminary ; it is the second method which is relied upon for obtaining photographic images of submicroscopic organisms by means of ultra-violet light. This also is a ‘ dark-ground ’ method, but it can be used either for visual observation or for taking ultra-violet photomicrographs. The dark-ground illuminator is made of quartz in its refracting elements and of magnalium in its reflecting surfaces. It will therefore transmit visible or ultra-violet light, and, by a simple changing mechanism, either can be used. Suitable material having been selected, by the visual method first described a preparation is made between quartz slide and cover glass, the image is focussed in visible light, and photographed in any suitable ultra-violet radiation, the necessary adjustment of focus being made in accordance with calculation.

“ A microscope of special design is used so that glass objectives can be used for all visual purposes and quartz objectives substituted without interference with object or image, for taking ultra-violet photo-micrographs.

“ For ultra-violet photography the source of light has been an electric spark-discharge between cadmium electrodes. By means of quartz prisms and lenses, essentially an ultra-violet spectrograph, the desired line in the cadmium spectrum is separated and projected on to the posterior surface of the quartz magnalium dark-ground illuminator referred to above. The wavelength commonly used has been 275 $\mu\mu$, but it is now possible to use shorter wavelengths over a considerable range. In this way it has been possible to obtain photographs with exposures so short as to reduce to a minimum the risk of change in the optical properties of the particles which are being photographed, through the action upon them of the ultra-violet rays.

" Microscopical preparations are made from filtrates, concentrates or centrifuged deposits. For visual examination a small drop of the fluid to be examined is placed between two thin cover glasses, the amount of fluid being such that none exudes from the edge of the cover glass even with slight pressure. A thin layer is essential to avoid interference arising from superimposed images, and to reduce the mean free path of the granules. For ultra-violet photography the layer must be as thin as practicable, but this is obviously in large part dependent on the viscosity of the fluid. As quartz slides and cover glasses, used for ultra-violet work, have optically worked surfaces, this is not so difficult as with glass, the fluid spreading more easily into a thin, even layer. Immobility of the granules must be secured before photographs can be taken. In part the thin layer secures this, but after a short lapse of time, usually an hour or two, the granules attach themselves to slide or cover glass. The best definition is secured when the granules are in optical contact with the under side of the cover glass, but, provided that the thickness of the layer is not more than two wavelengths, either surface can be used."

From a series of filtration experiments with his collodion membranes carried out in conjunction with Barnard's ultra-violet photography, Elford (4) calculated that the units of ectromelia virus have a diameter not greater than 0·15 μ or less than 0·1 μ. " For objects of this size, the use of rays of wavelength 0·275 μ for illumination should enable a true image to be obtained. Good, sharp photographic images were actually obtained, and measurements of them indicate a size within the range indicated by Elford, being, in fact, 0·13–0·14 μ." Barnard describes the organism so photographed as coccoid in form, pairs being common and elongation evident as a preliminary to division. " Isolated organisms are spherical and highly refractile, and the refractivity appears to increase with shortening of the wavelength employed for illumination. The small mass is apparently associated with high density, as indicated by the ease with which the bodies are thrown down by centrifugalization in narrow tubes. Reproduction is by binary fission, but the period of elongation, before division occurs, is short, the final separation taking place quickly and sometimes suddenly.

The separating elements often appear to have a connecting filament, which on rare occasions has actually been seen. The ultra-violet photographs suggest that there is a cell wall, indicated by a more highly refractive outline." Barnard points out, however, the possibility that, in cells so minute as these, the surface is so large in relation to the small mass that the cell wall may not be a separate structure, but only such a limiting membrane as would be produced by effects of surface tension.

By similar methods Barnard has also photographed living untreated vaccinia virus, in concentration from an emulsion of infected rabbit testicle (3). The image so obtained suggests the appearance of an organism.

Infra-red Photography of Virus Symptoms in Plants

It will be convenient to include in this chapter a short account of some recent work by Bawden (5) on the photography of virus symptoms in plants, more especially of the necrotic type, using infra-red plates and filter. In working with the " streak " diseases of the potato great difficulty has been experienced in obtaining adequate photographs of the necroses occurring in the dark-green leaf, by means of panchromatic plates and reflected

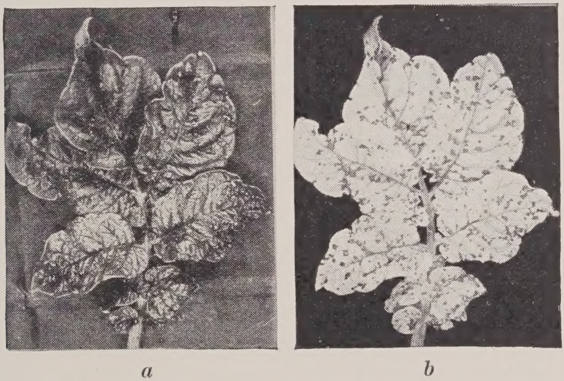

FIG. 39.—A necrotic potato leaf photographed (*a*) on a panchromatic plate, and (*b*) on an infra-red plate. (After Bawden.)

light. Necrotic leaves, unless the necrosis is extensive, photographed by this method usually give pictures of healthy leaves (Fig. 39*a*).

By the use of the new infra-red plates and filter, however, it has now been found possible to obtain photographs giving perfect contrast between the leaf and the necroses. The normal leaf cells completely reflect infra-red rays and in the positive are white, whilst the necrotic areas are jet-black (Fig. 39*b*). All the necroses produced in the potato, regardless of the causative virus, behave in the same manner.

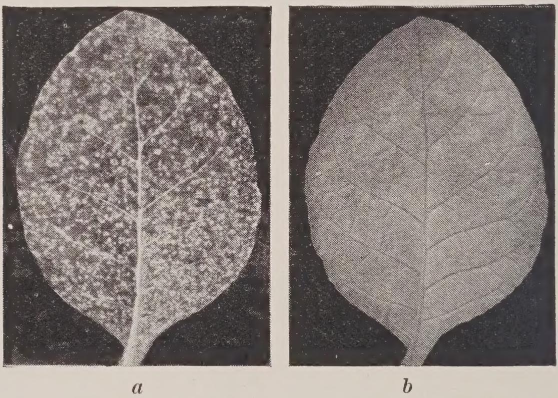

FIG. 40.—A tobacco leaf infected with a virulent strain of the X virus photographed (*a*) on a panchromatic plate, and (*b*) on an infra-red plate. (After Bawden.)

In photographing the necroses produced on tobacco by the X virus completely opposite results are obtained. The colour contrast between the leaf and necroses is great and they photograph well on panchromatic plates (Fig. 40*a*). In contrast, photographs of such necrotic leaves taken on infra-red plates present an appearance indistinguishable from that of healthy leaves (Fig. 40*b*). All mottles also behave in this manner and photograph well on panchromatic plates, but disappear entirely in infra-red pictures.

The explanation of these results seems to be that whereas the panchromatic plates differentiate only between colour differences the infra-red plates are capable of distinguishing between chemical differences. The potato necroses consist of dead cells, which, however, contain large amounts of breakdown products, rich in pectic substances and possibly tannins, and these completely absorb infra-red rays, whilst the X-type tobacco necroses consist merely of dead empty cells, *i.e.*, of cell walls only, which reflect infra-red rays in the same degree as normal cells.

Support for this view is obtained from the photographs of *Nicotiana glutinosa* infected with the X virus and tobacco suffering from tobacco ringspot. The colour contrast between the leaf and necroses in these is fair and all the necroses show in panchromatic photographs (Fig. 41*a*). With infra-red photographs only a portion of the necroses appear, but these show up more definitely (Fig. 41*b*). On examination these are found to contain degeneration products, whilst the remainder of the necroses consist merely of dead empty cells.

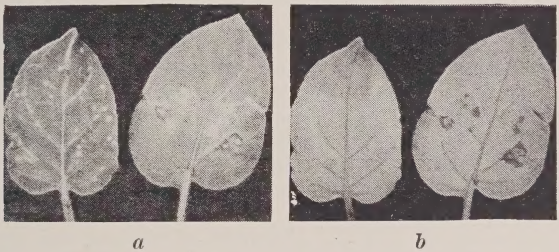

FIG. 41.—Leaves of *Nicotiana glutinosa* infected with a virulent strain of the X virus photographed (*a*) on a panchromatic plate, and (*b*) on an infra-red plate. (After Bawden.)

The infra-red plates are so sensitive to necrotic changes in the potato that clear photographs can be obtained of commencing necroses barely visible to the eye, and it seems possible that, in addition to the limited use of recording disease symptoms, infra-red photography may have a much wider application in the detection and differentiation of chemical changes occurring in the living plant.

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CHAPTER XI

SOME FURTHER ASPECTS OF THE STUDY OF PLANT VIRUSES (*Continued*)

Composite nature of certain virus diseases—Behaviour of viruses in a mixture—Variations in virulence—Mutations and variations in viruses—Methods of differentiation and classification of viruses.

Composite Nature of Certain Virus Diseases

The trend of recent research upon plant virus diseases has been to emphasize the possibility that in certain cases such diseases, especially in the potato (36, 46), are caused not by one virus entity alone but by a mixture of two or more such entities. The symptoms of the disease may be the result of the several viruses acting in unison, as in tomato streak, or they may be caused by one virus alone, the other virus or viruses playing no part in the production of the symptom picture. This latter state of affairs is frequently found in potato virus diseases.

It is proposed in this section first to describe shortly those diseases which have been proved, or are strongly suspected, to be complexes of viruses and then to outline the various methods which are available for demonstrating the composite nature of these virus diseases.

It is in the potato plant that the complex virus disease most frequently occurs, and this is due to two main reasons, first to the continuous propagation of the viruses by the tubers so that the potato stocks gradually become saturated with virus, and secondly to the ease with which certain viruses are " carried " without symptoms by a number of potato varieties. The existence of the virus thus carried may not be suspected until a scion of the carrier potato is grafted on to a non-carrying variety or until a second virus is added to the one carried when a disease such as crinkle is produced. It is not proposed at this juncture to describe these potato diseases in detail as they are so dealt with in Chapter XII. Suffice it to say that the work of the writer (46), of Murphy and M'Kay (36) and of others has revealed their composite nature, and the methods used to demonstrate this fact are outlined later in this chapter.

The tomato is another plant in which more than one virus frequently co-exist. In this country the most usual complexes are either mixtures of the different mosaic viruses or a mixture of mosaic virus and the virus of spotted wilt (46A). A virus complex commonly affecting the tomato plant in America produces a disease known as " streak." This disease has been shown by Dickson (8) and Vanterpool (54) to consist of a virus of the tobacco mosaic type mixed with a potato mosaic virus, probably the X virus of the writer (46). Tomato streak of this nature does not occur normally in England because the tomato crop is grown mostly under glass and opportunity for infection with a potato virus does not arise. Tomato streak produces a symptom picture somewhat similar to that of a tomato virus disease which does occur commonly in England, that known as tomato " stripe " (see p. 373). This disease may be due to a single virus of the tobacco mosaic type, as suggested by Jarrett (17), or it may be a virus complex. It is unfortunate that in her paper Miss Jarrett referred to tomato stripe as " streak." The two viruses producing streak in tomato will also produce a disease of the streak type when mixed in tobacco (40).

Valleau and Johnson (52) have experimented on the production of a tomato " streak " disease by mixing various viruses together in that plant. They produced streak by inoculation to tomatoes with three strains of true tobacco mosaic viruses plus a potato mosaic virus in each case. They also produced streak by means of three strains of cucumber mosaic viruses plus a potato mosaic virus, and similarly with three strains of an " etch " tobacco virus, so called. In the three virus groups studied, the one in each group producing the most severe symptoms on tobacco resulted in the most severe streak of tomatoes when combined with the potato virus. Valleau and Johnson also state that they were able to produce a streak in tomatoes by means of a single virus entity belonging to the group of tobacco mosaic viruses. This may correspond to the tomato " stripe " virus in England. The writer has produced similar tomato streak diseases by inoculation with the virus of a tomato mosaic and the X potato virus, and also by mixtures of various tobacco mosaic viruses and the X virus.

The raspberry plant is also frequently affected by composite virus diseases. Bennett (2) considers that the problem of the identification and classification of raspberry viruses is complex, and there is probably not enough information available to present a final verdict as to the number of distinct viruses involved. It is concluded that under Michigan conditions there are two general types of mosaic virus present in raspberries. The first produces " yellow mosaic," characterized by yellowing and stunting of the plants, and symptoms are not completely masked by summer temperatures. The second type, or " red-raspberry mosaic," is characterized by leaf mottling, by the production of necrotic areas in petioles and cane tips, and requires a low temperature for expression of these symptoms. It is possible that this latter virus really consists of more than one entity or it may be a single virus which varies in virulence. Combinations of yellow mosaic and red-raspberry mosaic often occur. In red varieties affected by yellow mosaic, mottling is accentuated by the presence of red-raspberry mosaic virus, even at temperatures too high for red-raspberry mosaic virus alone to cause symptoms. Cane striping is produced in black varieties by a combination of these two types of virus.

It seems probable that the transmissible variegation of tulips known as " breaking " may consist of two viruses. McKenny Hughes (30) describes two types of symptoms which he names " red break " and " white break "—" self " and " clear " breaks of Cayley (6)—and suggests that the former may be an earlier stage in the development of the virus while the latter is the final stage. McWhorter (35) considers that breaking is due to the interaction of two viruses. One of these viruses carries a colour-adding factor and produces no visible effect on leaves ; the other removes flower colour and strongly stripes the leaves.

The foregoing examples must suffice to indicate that the phenomenon of composite virus diseases in plants is by no means uncommon. It is indeed likely that improvements in the technique of virus study will reveal the existence of complexes in diseases where the presence of more than one virus entity has not hitherto been suspected.

A parallel case of a composite virus disease in animals may be quoted here. In studies upon louping-ill (an encephalomyelitis of sheep) it has been shown that ticks (*Ixodes ricinus*) which transmit the virus also harbour another infective agent which causes febrile disease in sheep. This is a distinct type of infection and is known as " tick-borne fever." It is clinically and immunologically distinct from louping-ill but may co-exist with it and aggravate the harmful effects of the latter virus (14).

There are several modes of procedure possible for demonstrating the existence of a virus complex in a plant virus disease and for separating out the component viruses. It can, however, only be shown by experiment which is the best method to use in a particular case. Analysis of a virus complex can be arrived at by taking advantage of differences in certain physical properties of the viruses which may exist, such as resistance to ageing and filterability, by using the power of selective virus transmission exhibited by certain insect vectors, by the use of differential hosts and occasionally by differences in the rates of movement of the respective viruses in the host. A few examples of the application of these methods may serve to make matters clearer.

The writer (46) has proved the composite nature of several potato mosaic diseases and the methods used for the analysis and synthesis of the diseases are briefly as follows. Separation was effected (1) by utilizing a selective relationship which exists between the aphis vector *Myzus persicæ* and the viruses causing the disease ; (2) by the use of what may be called " filter " plants, *i.e.*, plants which are susceptible to one of the component viruses but resistant or immune to the other ; (3) by taking advantage of the unequal rates of movement of the constituent viruses within the plant host (*a*) at the moment of development of primary symptoms in the young plant inoculated with the virus complex, (*b*) in the ageing plant. It may be of interest to describe an actual analysis and synthesis of a potato virus disease as frequently carried out, using the methods above described. So far as the writer is aware this was the first time a virus complex had been analyzed and synthesized in the living plant. The two viruses forming the complex are designated X and Y. The experiment was performed as follows, the source of infection being a mosaic-diseased Arran Victory potato plant. Needle inoculation

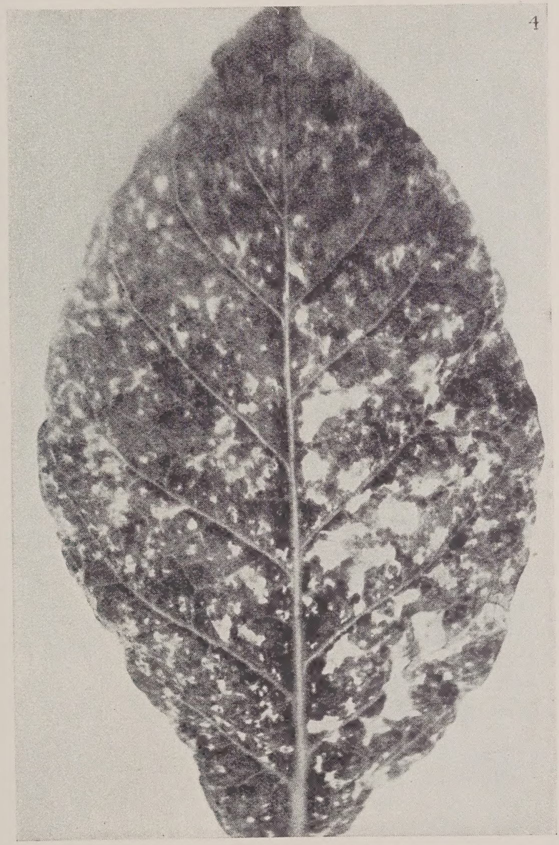

Fig. 42.—The potato viruses, X and Y, together on tobacco. Note the necrotic lesions and the increased severity of the resulting disease.

was made from this plant to White Burley tobacco seedlings; this resulted in the production of a virulent disease characterized by gross lesions and severe necrosis of the veins (see **Fig. 42**).

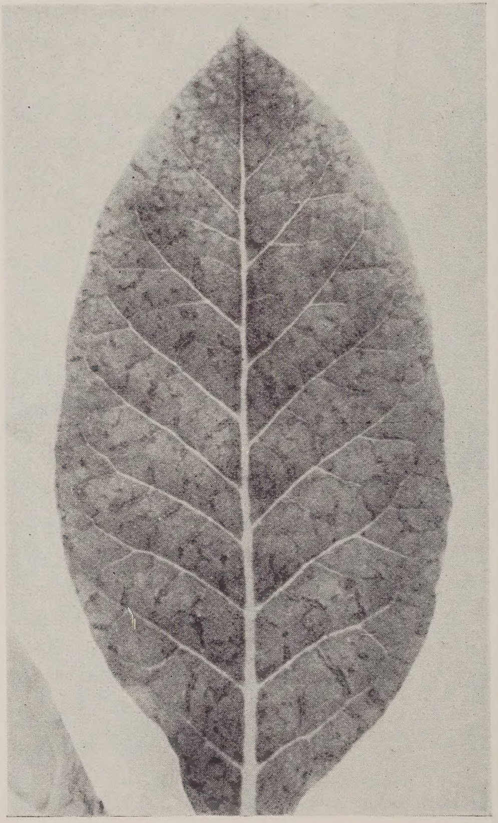

Fig. 43.—The Y potato virus on tobacco. Note the darkening of the tissue in the neighbourhood of the veins.

A parallel series of infection experiments was carried out from the same mosaic-diseased potato plant to White Burley by means of the aphis *Myzus persicæ* Sulz.; this resulted in the production of the disease due to virus Y, with its characteristic darkening of the green colour of the tissues along the veins (see **Fig. 43**). On passage of the necrotic complex through a " filter " plant, in this case *Datura stramonium*, back to tobacco again, the disease took on a "ringspot" form with numbers of clearly defined double concentric rings, but there was no general necrosis. This is a typical picture of the disease due to virus X (see Fig. 44). Now to a number of these tobacco plants showing the ringspot symptoms of X was added the virus Y, which had been isolated by means of the aphis. After the usual incubation period, the primary symptoms peculiar to the aphis-transmitted virus Y appeared;

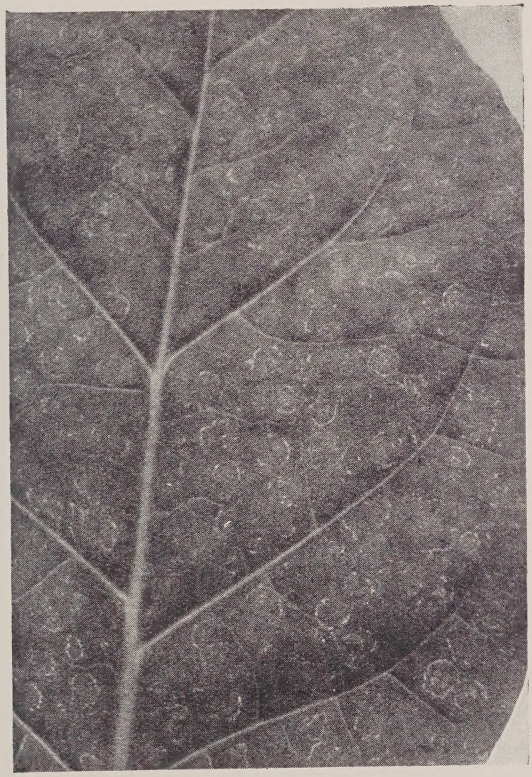

FIG. 44.—The X potato virus on tobacco. Note the numerous concentric rings.

following rapidly upon this, the rings lost their regular outline, became filled up and degenerated into irregular necrotic lesions, while a severe necrosis of the veins developed. In a short time the symptom picture was identical with that of the necrotic disease before the separation had been effected (see Fig. 42). Further passage through the filter plants again produced the ringspot disease X, while the addition to this of the aphis-carried virus Y once more restored the virulent necrotic disease.

The same analysis and synthesis can also be carried out by removing the X constituent from the complex by the substitution of an X-resistant " filter " plant for the Y-resistant *Datura stramonium.* This can be done by the use of *Petunia.* It should be pointed out, however, that *Petunia* is not immune to X as apparently *D. stramonium* is to Y. The resistance of the *Petunia* to X seems to depend largely on the particular strain of X used and also on the variety of garden *Petunia.*

Murphy and M'Kay (36) have demonstrated the composite nature of potato crinkle by synthetical rather than analytical methods and using potato plants only in their experiments. They introduced the virus of simple mosaic (= X virus of the writer) into Irish Chieftain potato " carrying " another virus, designated " A " ; this resulted in a disease indistinguishable from crinkle and which persisted as such. The work of Murphy and M'Kay is further dealt with in Chapter XII.

In experimenting with tomato plants affected with a " stripe "-like disease the writer demonstrated the composite nature of the affection, first by using the selective transmitting power of the insect *Thrips tabaci* which isolated the virus of spotted wilt, of which this insect is the vector, and secondly by filtration methods. Passage of the tomato juice through an L_3 Pasteur-Chamberland candle yielded a filtrate containing only the virus of a tomato mosaic, as the virus of tomato spotted wilt will not pass an L_3 candle. Differences in resistance to ageing effects in extracted juice were also made use of to separate these two viruses, the longevity of the virus of tomato spotted wilt in extracted juice being only four to six hours while that of the accompanying tomato mosaic virus is several months.

McKinney, by means of successive series of inoculations from the yellow and green areas in mosaic-affected tobacco plants, considers that he has differentiated the disease into component viruses causing respectively yellow or green mosaic diseases (33). He has obtained similar results by applying these methods to the differentiation of viruses causing green and yellow mosaics of wheat (34). Dufrenoy (9) states that he has confirmed McKinney's results " on the complex nature of viruses," by which presumably he means " virus diseases." He observed that juice from yellow areas of a mosaic tobacco leaf produced " yellow " areas where inoculated into mature leaves of tobacco. The juice from the " pale green " areas of mosaic leaves produces no local lesions at the inoculation point but induces typical green mosaic in the leaves subsequently developing. Similarly, McWhorter (35), in a preliminary analysis of tulip breaking, advances the theory that this transmissible variegation results from the interaction of two viruses. One of these appears to carry a colour-adding factor and produces no visible effect upon the leaves ; the other removes flower colour and strongly stripes the leaves. It was observed that certain varieties, when " broken," tend to segregate into plants or parts of plants bearing strongly darkened flowers or decidedly bleached flowers. By encouraging this segregation through selection and by inoculating from the parts of flowers where the colour was removed and darkened respectively, McWhorter considers it possible to secure the viruses in almost pure condition.

Behaviour of Viruses in a Mixture

The questions of how one virus influences another when two or more are co-existing in the same host, and whether there is any antagonistic or " cancelling " action between them, are of great interest. As a general rule it may be stated that the action on the host of two viruses acting in unison is productive of more severe symptoms than one virus acting alone. There are exceptions, however, notably in virus affections of the potato plant. As an example of this rule the disease complex tomato " streak " may be considered. The two viruses producing the disease, *i.e.*, that of tobacco mosaic and a virus of potato mosaic, do not singly produce a very serious disease in the tomato, but jointly they produce a severe necrosis frequently fatal to the young plant, and similarly with a mixture of the same two viruses in the tobacco plant. The potato viruses X and Y, when in combination, exert a much more deleterious effect on the tobacco plant than they do when acting singly (cf. Figs. 42–44).

Cases of symptom modification, not necessarily an increase in severity, induced by a virus complex are known. When the viruses of red-raspberry mosaic and yellow mosaic are mixed together in the same raspberry plant, symptoms of both viruses develop, but the former seems able to modify the symptoms of yellow mosaic on leaves produced at temperatures too high for red-raspberry mosaic alone to produce any effect (2). Another case of symptom modification induced by two viruses is shown by the interaction of the viruses of tobacco mosaic and ringspot in the tobacco plant. The symptoms of tobacco mosaic in tobacco plants which have recovered from an attack by, but still bear the virus of, ringspot are different from those in plants which have never been infected with ringspot. The plants carrying both viruses developed milder mottling symptoms than those infected with the tobacco mosaic virus alone (38).

McWhorter (35) states that he has observed an antagonistic action between the two viruses causing tulip " break," which he has called the " colour-adding " and " colour-removing " viruses. The latter virus alone is extremely destructive, and is only with difficulty maintained in " pure culture " because of this fact. The former virus, which is so powerful in its action that it is possible to turn a white tulip bright red or a pink tulip vermilion, produces these colour reactions and affects growth to a very limited extent when a trace of the other virus is present. When this trace of the other virus is not present and the colour-adding virus is pure, it becomes very virulent and has a pronounced burning effect upon certain tissues. From these facts McWhorter deduces an antagonistic reaction between the two viruses. See also Thung, p. 247.

There seems to be a certain amount of evidence that the addition of a virus causing chlorosis, to a hop plant which is a symptomless carrier of mosaic, induces marked symptoms of the latter disease. It must, however, be noted that two cases of a natural appearance of mosaic disease in hop plants hitherto symptomless carriers have also been recorded (43). This is the opposite effect to that claimed by Salaman (41), who states: (1) that a virus complex is not a mere mixture but is a " linked group "; (2) that the phenomenon of auto-infection occurs in potato plants; and (3) that it is possible to produce artificially, symptomless " carrier " potato plants. It is clear that the phenomenon of auto-infection and the production of carriers are dependent upon the ability of separate viruses to form a " linked group." In other words, the spontaneous breaking down of a virus " compound " produces " auto-infection " by the liberation of a " loose " virus within the plant, while, conversely, the linking together within the plant of two viruses, each of which independently produces symptoms, may lead to the formation of a " linked group " which is carried without symptoms in the host plant. It is not quite clear what is meant by a " linked group which is not a mere mixture." Salaman (41, p. 222) implies that it does not infer anything comparable to a chemical compound, although a later paper (42) appears to treat this " linked group " as a stable entity, for on p. 65 it says, " it is clear that this virus complex does not readily break up even in the foreign environment of a different species, such as tobacco," and on p. 70 of the same paper, " an otherwise uninoculable complex must suffer disintegration on contact with the strange cytoplasm before one or more of its constituents can act." The writer's experience of mixing viruses in the potato plant has led him more to the conclusion that when two viruses are thus mixed the symptoms of both develop but the more severe disease tends to obscure the symptom picture of the less severe disease. The potato virus Y which produces leaf and stem necrosis in a number of potato varieties, produces these symptoms irrespective of the presence of one or more mottle-producing viruses.

As regards the behaviour of mixed viruses in the animal host, evidence suggests that in sheep the virus of tick-borne fever may have an important influence on the course of the disease induced by the virus of louping-ill. In the absence of tick-borne fever the typical nervous symptoms of louping-ill are unlikely to develop, although infection with the virus of the latter occurs (29).

During recent years it has been suggested that infection with one virus, *e.g.*, that of vaccinia, might perhaps awaken into activity a latent infection with a hitherto unrecognized virus (cf. above: the behaviour of the hop plant carrying mosaic infected with the virus of a chlorotic disease), or, alternatively, modify the character of the lesions produced by a previously known virus. It appears, however, that infection of monkeys with a highly virulent vaccine virus (neuro-vaccine) has no power to modify the nervous lesions of poliomyelitis. In short, no definite evidence has been brought forward that under any circumstances can the poliomyelitis virus evoke lesions other than those usually associated with its activity (16).

Variations in Virulence

There is fairly good evidence that certain viruses may vary in degree of virulence, such variations in virulence being measured by the response of the host and by dilution tests. Attenuation in a virus is indicated by reduction in the severity of the host symptoms and a diminution in infective power. It is best measured by means of dilution tests. Variations in virulence can be brought about in different ways: by passage of resistant or susceptible hosts, by treatment of the virus with physical or chemical agents and possibly by ageing *in vitro*. The best example of changes in virulence of a virus induced by passage of various species of plants is given by certain experiments with the virus of curly top of the sugar beet. It has been found that passage of normal virulent curly top virus through individual resistant sugar beet plants results in attenuation of the virus (26). The variation in degree of attenuation appears to correspond directly to the severity of the symptoms on the infected resistant plant used as a source of the attenuated virus. This attenuation of the virus by resistant sugar beets has been demonstrated for three different virus strains. It appears that neither prolonged development of the attenuated virus in a susceptible plant nor repeated passage of it through susceptible plants restores its virulence (5). It has also been demonstrated that passage of the weed *Chenopodium murale* attenuates the curly top virus (4). Further investigation has revealed that other plants, both wild and cultivated, have the power of attenuating this virus. The following have this effect: lamb's quarter, *Chenopodium album* and *C. album viride*, tomato, and peasants' tobacco, *Nicotiana rustica* (27). Virulent virus of sugar beet curly top, attenuated by passage of *Chenopodium murale*, can be restored to its original virulence by passage of chickweed, *Stellaria media*. Experimental evidence indicates that the attenuation is due to a change in the quality rather than in the quantity of the virus involved. Virus which has been attenuated by passage of *Chenopodium murale* appears to be quite stable and cannot be restored to virulence by passage of very susceptible sugar beets. It must be passed through a particular plant host such as *Stellaria media* in order to become reactivated. This plant has no effect upon the virus in its virulent condition. It has been found that the incubation periods in the plant of the virulent and restored forms of the virus were practically of the same length, but the attenuated virus had a much longer incubation period (28). Although there does not appear to be another case of attenuation and restoration of virus virulence so clear-cut as the foregoing, yet there are other instances where passage through, or sojourn in, particular plant hosts appear to increase virulence. The writer has shown that the potato virus X tends to increase in virulence by progressive passage through susceptible varieties of tobacco (45). It is not yet certain whether the different symptoms occurring in raspberry plants affected with mosaic, and designated mild, medium-severe and severe red-raspberry mosaic, are due to several viruses or to one virus in differing degrees of virulence, and it must be left to future research to perfect a method for differentiation and identification (2).

Johnson (18) has shown that the American " healthy potato virus " apparently changes in virulence, and the same worker (19) has attenuated the viruses of certain tobacco mosaics by means of heat. Plants soon after inoculation were placed in constant temperature chambers at a temperature between 35° and 37° C. for ten or more days. At this temperature the symptoms are wholly or partially masked. Transfers of virus from such plants in comparison with that from plants unexposed to heat shows that a very decided attenuation has occurred. The attenuated virus usually gives only mild mottling and little or no malformation as compared with the masked symptoms of tobacco mosaic. Apparently this attenuation can be obtained in varying degrees, depending upon the temperature exposure within certain limits. The attenuation of the tobacco mosaic virus described by Johnson appears to be fairly stable as the altered virus can be repeatedly transferred serially through tobacco plants without changing the attenuated condition.

In experiments with the virus of tomato spotted wilt the writer has observed what appears to be an interesting change in virulence. The particular strain of this virus was obtained four years ago, occurring naturally in a plant of *Solanum capsicastrum*. On transfer to tobacco, the symptoms produced by this virus were of a ringspot character, numbers of concentric rings developing on the leaves. As a rule the effect of the virus was not very serious. Gradually, however, with long sojourn in tobacco plants, the ringspot type of symptom in this plant has disappeared and symptoms take the almost invariable form of a fierce, general and scorching necrosis. The death-rate of tobacco plants affected with this type of symptom is 100 per cent. As the environmental conditions and tobacco varieties are the same as in 1929, this symptom change and great increase in killing power of the virus may be legitimately attributed to an increase in virulence which appears to apply particularly to the tobacco plant.

McKinney (32) noticed that in some cases an increase in potency was found in the virus of tobacco mosaic on the sixth and twelfth days after dilution, but on standing fifty days there was a decrease of potency below that of any previous point. Similarly, Olitsky and Forsbeck (37) state : " In nearly every case, irrespective of the method employed, there was some increase of the potency of the virus beyond the original titration." The writer has also observed an apparent increase in virulence in a sterile L_3 filtrate of the potato virus X, after standing for seven days. It is not known to what this apparent increase is due, but there is no evidence that there is any multiplication of the virus agent *in vitro* (see p. 254).

As regards variation in virulence of animal viruses, it has been shown that the virus of pleuropneumonia of cattle becomes attenuated on keeping and can be used for producing immunity (1).

The virus of human poliomyelitis increases in virulence on passage through a susceptible host to which it has not hitherto been adapted. This virus, when fresh from the human subject, kills only 20 to 40 per cent. of monkeys, but after passage of a number of monkeys the mortality rises to nearly 100 per cent. (13).

Mutations and Variations in Viruses

In the foregoing paragraph evidence is presented which indicates that in certain cases plant viruses can be attenuated. The differences which then exist between a virus and its attenuated strain refer only to the symptom picture ; so far as is known it is not possible to produce by attenuation alone differences in host range or variations in insect vector relationships. In the present section it is proposed to discuss the possibility of strains of viruses developing or " mutating " from a particular virus in such a way that slight but definite differences in character emerge.

Perhaps the best illustration of the existence of two apparently independent strains of a plant virus is afforded by the case of aster yellows and celery yellows. Severin (44), working with the virus of aster yellows in California, found it easy to transmit the disease by means of its vector, the leaf hopper *Cicadula sexnotata*, to celery, while Kunkel (23), working in New York with a local strain of aster yellows, found that celery was apparently immune or at least highly resistant to the virus. Kunkel obtained from Severin a sample of the Californian aster yellows and found that it was transmissible to celery under New York conditions, while the local strain under similar conditions was not transmissible to celery. In every other respect of symptoms, incubation period in the plant and identity of insect vector the two viruses are identical (24).

Here then is apparently a case of a virus having " mutated " or adapted itself to a new host plant in one district and after sojourn in this host has acquired the ability to infect it as easily as any other plant in its host range. Such a virus may be regarded merely as a slightly different strain of aster yellows, or it may be regarded as a different entity and be referred to as " celery yellows." It is also possible that celery yellows is a stage in the evolution of an entirely new virus. In the writer's opinion slight differences in host range do not justify the separation into distinct viruses of entities which are otherwise identical. A possible parallel case to the foregoing may be found in the dissemination of the virus of the sugar beet curly top by *Eutettix tenellus* in the U.S.A. and by *Agallia sticticollis* in the Argentine. Here may be a case of a virus mutating in regard to its insect vector relationship. Similarly, the potato virus X, after long sojourn in the tobacco plant, is returned to the potato with difficulty.

There appear to exist several very similar viruses affecting the raspberry and its varieties in America which may perhaps be legitimately described as " strains." Thus there are two separate kinds of streak in black raspberries : one is designated " mild streak " and the other " severe streak." Several of the symptoms of mild streak indicate a close relationship with severe streak. The same typical recurving and hooking of the midribs of tip leaflets is a symptom common to both diseases, and in both it is identical in appearance and extent. A general rosetted appearance of affected plants is noticeable in both diseases, but since the growth is not stunted much by mild streak the rosetting is less pronounced. Despite these similarities, mild streak may be distinguished from severe streak by certain symptom differences. A conspicuous and distinctive blotching or gross mottling of the older foliage is present in all fully developed cases of severe streak, this character being absent or faint in the mild type. The streaks themselves are also different : broad, short and dark blue or brown in the severe ; slender, long and light blue-grey in the mild. Stunting is produced by severe streak but not by mild, and the fruit is affected by mild streak but not by severe (7).

Similarly, Bennett (2) describes three types of red-raspberry mosaic which he designates mild, medium-severe and severe. He states (loc. cit., p. 31) : " While it is by no means established that only one virus is responsible for the production of this range of symptoms, it seems probable that such may be the case and that the range of severity of symptoms may be produced by strains of the virus in different stages of virulence." It cannot be said whether the difference between these strains is one of virulence only or whether other differences also exist.

Further evidence of the existence of strains of viruses is to be found in the " streak " diseases affecting maize and sugar cane (49). The virus of streak disease of maize, as it usually occurs in the field, is not identical with the virus of streak of Uba cane. Each virus shows a specialization to its own host. The maize virus produces a severe disease in maize and is only weakly virulent to sugar cane, while the Uba virus which produces a mild though permanent disease in Uba cane is weakly virulent to maize. A species of wild grass, *Digitaria horizontalis*, appears to be susceptible to a virus which, while similar, is not identical with either the cane or the maize virus. The trend of this work of Storey and McClean (49) has been to show that there is not one streak virus but several which may be regarded as distinct entities or, preferably in the writer's opinion, strains of one virus specialized to particular hosts. Although at the moment definite evidence may be opposed to the idea that one streak virus may be changed into another, it can hardly be disputed that the streak viruses form a natural group, clearly differentiated from the mosaic viruses affecting a nearly similar range of host plants. The first signs of the streak diseases in their hosts are all similar, viz., discrete chlorotic areas situated along the veins and the insect vector is the same in every case : *i.e.*, *Cicadulina mbila* N. Storey and McClean say : " It is difficult not to believe that in the course of years there has evolved in Natal, perhaps from the maize virus, a strain of streak virus strongly virulent only to Uba cane." Stahl (47) has described a disease of maize in Cuba which is symptomatically similar to the streak disease described by Storey, but which has another insect vector, *Peregrinus maidis.* This insect was unable to transmit Storey's streak virus. This may possibly be a case of a virus having become adapted to another insect vector, or, as Storey suggests (48), the two diseases might be due to " strains " of a virus differing in their ability to come into association with *Peregrinus maidis.*

Instances of adaptation and variation in animal viruses are not wanting, the classic example being the adaptation of smallpox virus in calves. When smallpox virus has been passed through a sufficient number of calves in succession, or even through rabbits only, it becomes " vaccinia," and does not, even after repeated passages in man, return to its original condition (50).

The pox diseases generally are of interest from this point of view. While fowl-pox is probably due to a separate virus, the animal poxes (sheep-pox, goat-pox, swine-pox, horse-pox and camel-pox) are probably very closely related in the sense of being modifications of one virus. Inoculation of any of the group into the rabbit tends after passage to give a disease like vaccinia, which is perhaps to be regarded as the basal, least-elaborated member of the group. It seems clear that vaccinia never undergoes the reverse modification back into smallpox, although reversion of the virus to full human pathogenicity has been stated to occur on passage through the human body, but only if the virus is recovered in the earliest stages of calf-passage (13).

Immunization of poultry against fowl-pox can be carried out with pigeon-pox virus, which is probably very closely related to it (1). Some recent work by Burnet and Macnamara (3) has demonstrated the existence of immunological differences between strains of poliomyelitis virus. Results of the experiments show clearly that paralysis by poliomyelitis is not necessarily indicative of complete resistance to subsequent infection, provided that the second infection is with a virus of greater virulence or different type from the first. The question arises as to whether the differences between viruses are sufficient to justify differentiation into immunological types corresponding, for example, to the " *A* " and " *O* " types of foot-and-mouth disease. Evidence is given which clearly shows that infection and paralysis with the Australian virus usually does not protect against the American virus. There seem to be definite minor immunological differences in the two strains used. The virus of foot-and-mouth disease when taken direct from cattle, sheep or swine is much less infective for guinea pigs than the same strain after it has been adapted to guinea pigs by several passages. Vallée and Carré (53) have shown the existence of two types of foot-and-mouth disease virus which could be distinguished by a failure of the state of immunity towards one to protect against the other. It has now been shown that there are three types of this virus which are designated " *A* " and " *O* " (Vallée and Carré) and "*C*" (Waldmann). There is no indication that a strain of virus alters in type by passage through another susceptible species, but clear-cut differentiation of types can only be expected if attention be paid to the virulence of the strain examined and the degree of immunity of the animals employed (50, p. 60).

Methods of Differentiation and Classification of Viruses

It has been the ambition of many plant virus workers of recent years to evolve a stable and comprehensible means of differentiating and classifying the large number of plant viruses now known to exist. Various methods of classification have been suggested and these will be briefly discussed. Much of the confusion at present existing in the study of plant viruses is due to the fact that writers have attempted to name the disease instead of the virus, and also because they have not kept clear in their minds the distinction between the disease and its causative agent. The following are cases in point: Folsom (12) states: "Not only does the same *virosis* have different symptom aggregates, but, with a close relationship between some causal agencies apparently existing, the same symptom aggregate sometimes is ascribable to several *viroses*." If, as Folsom states, virosis is a name for " virus disease," or in other words the symptom picture, it is manifestly incorrect to speak of a particular set of symptoms having different symptom aggregates or being ascribable to several *viroses*. Similarly Hamilton (15), referring to the writer's X and Y viruses, speaks of them as " crinkle X " and " crinkle Y," whereas " crinkle " is the name of a *disease*—composite in nature as it happens—and not a *virus*.

The time has now come to attempt to name the virus itself and not the disease it causes, which may differ according to the host on which it happens to be discovered. Owing to the wide range of symptom expression on different hosts a single virus may be—and certainly has been already in some cases—described several times under separate names by different workers. This difficulty would be obviated if the virus itself were to be considered.

The various methods of classification already suggested have been based upon one or other of the following reactions :—

(1) Reactions of the virus with physical or chemical agents.
(2) Symptom range on differential hosts.
(3) Pathological histology.
(4) Relationship with insect vectors.

Each of these methods has some advantages, but each has also serious disadvantages. The first method, that of the reaction of the virus with physical and chemical agents, has been developed by Johnson (20). The chief tests applied by him were longevity *in vitro*, thermal death-points and lethal action of chemicals. The symptomatology on differential hosts was also studied. By these methods Johnson has shown the existence of eleven different viruses on tobacco and related plants which he has named numerically. In his latest paper, however, Johnson has not referred the potato viruses to numbers or letters, but still describes them as various types of mosaic or crinkle. The main disadvantage of studies on physical properties of viruses as a means of differentiation lies in their limited application. Such studies are, of course, only applicable to sap-inoculable viruses, and there is a large group of plant viruses only transmissible by insect vectors. It is true that developments in the technique of feeding insects on virus solutions (see Chapter V.) may allow, to a limited degree, of the study of the physical properties of viruses which are only insect-borne, but considerable time must elapse before this can become generally applicable.

The symptom range of viruses on a series of differential hosts has also been studied by Johnson, and this method forms a good adjunct to the studies of physical properties. The pathological histology of a virus-diseased plant has been used by Quanjer (39) as a basis for the differentiation and classification of potato viruses and this work is further considered in Chapter XII. Suggestions that the insect relationship of plant viruses might be used to classify the viruses have been made by Elze (10) and Storey (48). Elze's classification, however, consists only of a loose grouping of the viruses without any practical application. He divides them into three groups : those in the first group are linked together by the rather negative qualification that their insect vectors are not known. This group is likely to be reduced in numbers by the progress of research ; indeed one of the virus diseases included in this group, peach yellows, must now be excluded on the ground that the vector is discovered (25). Group 2 contains those viruses which are spread by different insects and are not specially adapted to any of these insects. Group 3, which contains viruses adapted to particular insects, is subdivided into sections, firstly *A* containing viruses which, in addition, are transmitted mechanically by other insects. Elze gives as an example of this group the virus of potato leaf roll, which he considers is transmitted by a flea beetle " only accidentally through virus residue sticking to the mouth-parts." As the virus of potato leaf roll is not transmissible by any kind of mechanical inoculation it is difficult to understand how it can be transferred mechanically on the jaws of the beetle. Secondly, there is section *B*, containing viruses with a specific insect vector, and this section is again divided according to the length of the " incubation period " of the virus in the insect vector.

Storey discusses the bearing of insect vectors on the differentiation of plant viruses and considers it justifiable to regard the insect as being infected by the virus no less than is the plant. It is true, as he points out, that a grouping of viruses upon the basis of the type of symptom manifestation would agree with a grouping upon the type of insect vector. Thus, there is a group of mosaic viruses transmitted for the most part by aphides and the yellows group transmitted by Jassids. It is interesting to note that evidence is beginning to reveal a homogeneous group transmitted by whitefly (*Aleyrodidæ*), and there may possibly be a thrips-transmitted group as well. The disadvantages to a classification of viruses based on insect relationships are firstly the fact that some insects transmit many viruses, *e.g.*, *Myzus persicæ* is the vector of thirteen viruses, and secondly, the possibility that the relationship between a plant virus and a specific vector is not stable.

While most of the methods of virus differentiation above outlined are good, yet in themselves they are not sufficient. The first step towards a stable classification is the collection of as much information as possible about each virus, and not the disease only, by the following methods : (1) physical properties where the virus is sap-transmissible, (2) (*a*) host range and symptoms, particularly the symptom expression on differential hosts ; (*b*) pathological histology and cell inclusions ; and (3) modes of transmission. It is thus probably only a matter of waiting until sufficient information can be collected concerning each separate virus. One can then look forward to a time when a standard series of tests will be evolved to which each apparently new virus can be submitted and its reactions compared with those of known virus entities.

One or two tentative schemes of classification of animal viruses have been put forward. McKinley (31) presents a classification of virus diseases based upon the presence or absence of inclusion bodies, transmissibility of the virus and filterability of the infecting agent, while Fine has drawn up a tabular presentation of viruses achieved primarily on epidemiological and pathological grounds (11).

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CHAPTER XII

POTATO VIRUS DISEASES AND VIRUSES

Potato virus diseases other than the mosaic group—Potato mosaic diseases.

This chapter is divided into two sections: the first is devoted to short descriptions of various potato virus diseases other than those of the mosaic group, in which the most important known facts concerning these diseases are given. The second section deals only with the potato mosaic group, description of these diseases and some properties of their causative agents are given and an attempt is made to clear up some of the confusion existing in regard to the study of this difficult group of viruses.

It may be pointed out that only viruses normally attacking the potato plant are dealt with here: viruses which can be induced experimentally to attack the potato plant, or do so only rarely under natural conditions, are listed at the end of this chapter; further information on these particular viruses will be found in Chapters XIII. and XIV.

In the descriptions of the various virus diseases and their causative agents in this and the two succeeding chapters, information on the following points is given wherever possible: the most typical symptom picture, modes of transmission and identity of insect vectors, the pathological histology and intracellular inclusions, any physically and chemically determinable properties of the virus *in vitro*, and finally the geographical distribution.

Potato Virus Diseases other than the Mosaic Group

Potato Leaf Roll. This is one of the few potato virus diseases which are fairly easily diagnosed on a symptom basis alone, the main symptoms, starch accumulation and rolling of the leaves, being more or less constant though the degree of rolling differs in some varieties. There are two symptom phases, primary and secondary leaf rolling—in the former only the topmost leaves, show a stiff upward rolling, which develops progressively to the lower leaves. In plants grown under glass, and in certain varieties, this primary phase is very transitory. In secondary leaf roll, which shows in all potato varieties the year following that of infection, the lowest leaves become thickened and leathery and begin to roll upwards at their edges, the roll commencing at the base of the leaf while there is a considerable accumulation of starch in the leaves. The thickening and rolling of the lowest leaves is a constant external symptom of the disease. As already mentioned the degree of rolling may vary; in such varieties as Arran Victory and Edzell Blue the rolling is very pronounced, accompanied by considerable pigmentation in the leaves of the former variety (see Figs. 45, 46). In President the leaves develop a stiff upright habit without much rolling and become yellow and harsh with some red pigment at their bases (see Fig. 47).

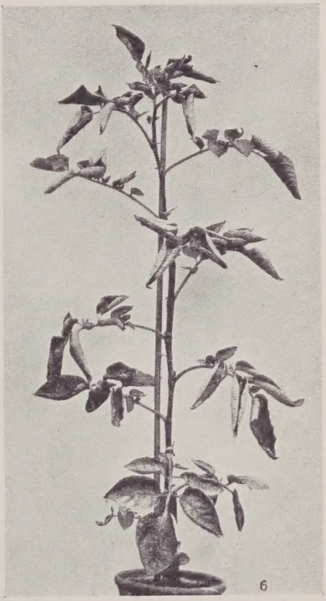

Fig. 45.—Potato leaf roll, var. Arran Victory. Note the pronounced rolling of the leaves.

The virus of leaf roll is not sap-transmissible, but can be conveyed by grafting of the stem and, rarely, by tuber core-grafts (25). As regards the insect vectors of this virus, there exists considerable disagreement among potato virus workers as to their identity, and the writer gives the following aphides as being in his opinion the chief, but possibly not the only, vectors of potato leaf roll: *Myzus persicæ* Sulz., *Myzus pseudosolani* Theob. and *Myzus cir-*

cumflex Buckt. (49, 52A). Of these three the first-named is the most important and efficient vector.

The chief histo-pathological symptom is a thickening of the walls of the primary phloem groups, which may be sufficiently severe to cause obliteration of the sieve tubes. In European potatoes this phloem necrosis appears to be restricted to the stems and leaves, but in certain American varieties, notably Green Mountain, a phloem necrosis occurs in the tuber as a current-season symptom and is known as "net necrosis." No intracellular inclusions have been observed in association with this disease. Owing to the fact that the virus is not sap-transmissible, studies on its physical properties have not yet been carried out. The disease of leaf roll appears to be widely spread over most of Europe and the United States of America. The writer has received specimens of it from Australia, Tasmania and Rhodesia. It probably occurs wherever the potato is grown.

References. Smith, K. M. (47, 49), Murphy, P. A., and M'Kay, R. (25A), Whitehead, T. (52A), Quanjer, H. M. (31), Gilbert, A. H. (13).

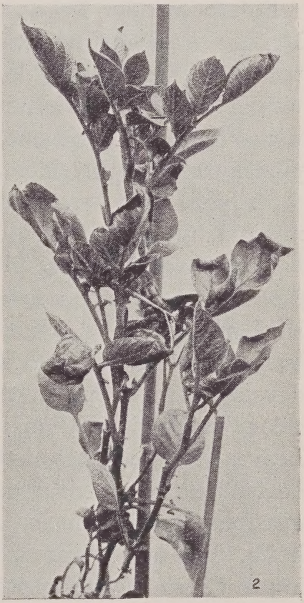

FIG. 47.—Potato leaf roll, var. President. Note stiff upright habit, leaves not markedly rolled.

Apical Leaf Roll of Potato. Schultz and Bonde (39) describe a new virus disease of the potato, so far only observed in America. This disease, called apical leaf roll, manifests itself in slight dwarfing of the plants with a roll of the upper leaves similar to primary leaf roll. The rolling, however, persists, in succeeding generations, in the upper leaves only and does not spread to the lower leaves as in ordinary leaf roll. The tubers, reduced in size but normal in shape and number, are frequently but not invariably attached to short stolons resembling the rather clustered tuber set of witch's broom plants. Apical leaf roll plants can be distinguished from those affected with witch's broom by taller, more vigorous and fewer shoots which form larger and fewer tubers. The virus is perpetuated by the tuber and can be transmitted to healthy tubers by core-grafts.

Potato Spindle Tuber. The American potato variety, Green Mountain, affected with this disease produces small upright plants, not straggling, leaves dark green or greyish in colour without visible mottle, smaller, more erect and more rugose. Leaves wither gradually without yellowing and remain hanging on the plant. Infected President plants are upright and dwarfed, about 6 in. high, with a red roll in the top like primary leaf roll, and marked by the same peculiar dying of the leaves from the top backwards and from the base of the plant upwards. The tubers, especially long tubers like the American varieties Netted Gem or Burbank, are of an abnormal spindle shape with an irregular or bumpy outline. Cylindrical tubers become more rectangular and develop deep eyes. In the case of Bliss Triumph a lighter red colour is obtained than in healthy tubers of the same variety. Leaves are sometimes narrower than normal and the petioles may be twisted. The virus of this disease appears to be sap-transmissible, by leaf mutilation and by means of the tuber-cutting knife. It is also transmissible by core-grafting the tuber. According to Goss (14) the insect vectors are grasshoppers (*Melanoplus* spp.); the flea beetles (*Epitrix cucumeris* and *Systena tæniata*); the tarnished plant bug (*Lygus pratensis*); the larvæ of the Colorado beetle (*Leptinotarsa decemlineata*) and the leaf beetle (*Disonycha triangularis*). Folsom states (11) that aphides (unspecified) also transmit the virus.

No studies of the pathological histology of this disease in the leaves appear to have been made. The virus of spindle tuber withstands dilutions of 1/1000 but shows a great falling off in positive infection above that dilution (14). The thermal death-point appears to lie between 60° and 65° C. (38).

The disease of potato spindle tuber has not been recorded in the British Isles and, so far as the writer is aware, not in Europe. It is prevalent in the United States of America.

References. Murphy, P. A., and M'Kay, R. (27), Young, P. A., and Morris, H. E. (54), McKay, M. B., and Dykstra, T. P. (22).

Unmottled Curly Dwarf. The symptoms of this disease are very similar to those of spindle tuber and the two diseases are thought by some workers to be the same. There is, however, a certain amount of evidence which tends to prove that the two disorders are distinct. According to Burr (7) a differential symptom is the development in unmottled curly dwarf of internal necrosis of the tubers in the current season. Tubers from the varieties Earliest-of-all and Bliss Triumph, which were severely affected with unmottled curly dwarf, were not elongate with too many eyes, the symptoms of spindle tuber (54). Goss (14) differentiates between the two diseases chiefly by the absence in spindle tuber of downward curving of the leaf along the midrib, rigidity and brittleness of stems and petioles. These symptoms are pronounced in unmottled curly dwarf. There do not, however, appear to be any very rigid distinctions between the two, and there is disagreement among different workers ; thus Goss says that the increase in the number of eyes in the tuber is extreme in unmottled curly dwarf while this symptom is given by Young and Morris above, as a characteristic of spindle tuber. The insect vectors of unmottled curly dwarf as given by Goss are the same as for spindle tuber with the exception of the leaf beetle (*Disonycha triangularis*).

There is no information on the pathological histology of the disease. The virus appears able to stand a temperature of 75° C., which is 10–15 degrees above the thermal death-point of spindle tuber as given by Samson (38). The difference in the technique employed in the respective tests, however, does not allow of a fair comparison. The disease appears to be fairly widespread in parts of the U.S.A., especially in Colorado and Nebraska ; there are no records of it in Europe.

Potato Witch's Broom. This American disease is considered by Murphy and M'Kay (27) to be the same as that first described in Scotland as wilding or semi-wilding. The affected plant is slow to come up and produces many weak shoots, bearing leaves of thin texture and light-green colour, which are either simple, or reduced compound leaves with round or heart-shaped leaflets. Another feature of the disease is the outstanding number of very small tubers formed, sometimes as many as one hundred or more. A common character of this virus in Arran Victory and President is the premature sprouting of the tubers during the autumn, with production, from the eye, of spindling shoots, which send out further lateral shoots from their basal portions (27). The potato virus disease formerly known as " spindling sprout " is not a separate entity and is merely one characteristic of witch's broom. The following are the symptoms of witch's broom as given by Young and Morris (53)—the primary symptoms are: dwarfed, abnormally light-green or yellow (= flavescent) top leaves, often with yellow margins; upper parts of stems cylindrical, with swollen nodes; rapid elongation of stems; profuse branching of the tops; aerial tubers; basal sprouts from tubers; and numerous small subterranean tubers. These symptoms intergrade with the secondary symptoms, which are flavescence and purpling of tops; marginal flavescence and dwarfing of the leaflets; simple leaves; numerous spindling axillary branches; slender cylindrical stems with enlarged nodes; filamentous stems; spindling sprouts from tubers or the base of the stem. The virus of witch's broom is transmissible by grafting and by core-grafting of the tubers. It is not apparently transmissible by sap-inoculation and the insect vectors are not known. There is no information on the pathological histology of the disease. It occurs in Europe and the U.S.A.

References. Hungerford, C. W., and Dana, B. F. (17), McKay, M. B., and Dykstra, T. P. (22), Schultz, E. S., and Folsom, D. (40, 41).

Giant Hill. An affection, known in America as " Giant Hill," resembles the condition known in Scotland as " bolting." The symptoms consist in the production of abnormally large and coarse haulms, with irregular tubers which may be cracked in many cases. In Green Mountain the plants are tall with dark-green leaves and stems unusually red. It is not yet certain whether this condition is a virus disease or a genetic abnormality.

Various attempts to transmit Giant Hill by grafting and insects have failed, though Dana (9) states that he has transmitted Giant Hill in one inoculated plant by core-grafts.

References. Murphy and M'Kay (27), Young and Morris (54), McKay and Dykstra (22).

Potato Calico. Leaflets of infected plants are irregularly spotted or blotched; these areas do not become necrotic but appear devoid of chlorophyll and generally assume a bright brilliant yellow, yellowish-white or grey colour. The spots are not always interveinal and may occupy as much as 95 per cent. of the leaflet area, being, as a rule, irregularly scattered. Plants, when infected young, seldom attain normal size. The virus is sap-inoculable, but is doubtfully transmissible by core-grafting; it does not appear to be filterable through a candle. The insect vectors are not known (29). Young and Morris (54) could find no abnormalities in sections of typically affected leaves. The disease appears to be confined to America.

Leaf Rolling Mosaic. The following are the symptoms of this disease as described by Schultz and Folsom (40): slight dwarfing, diffused mottling, wrinkling, slight ruffling and rolling of the upper leaves. Tubers show a general average reduction in size. On the American potato variety, Green Mountain, leaf rolling mosaic is a very serious disease. The plants are curly dwarfs, with streaking in the veins and petioles, later the leaves drop and the plants become straggling (27). The following description of leaf rolling mosaic is given by McKay and Dykstra (22). The mottling of the leaflets is diffused and resembles the type found in rugose mosaic. The leaves generally show a rolling upwards but are flaccid and the rolling resembles that type found in plants affected with Rhizoctonia or black-leg. This complex of symptoms, the rolling and the mottling, according to McKay and Dykstra, is apparently due to a single virus and as such is always transmitted. There is considerable difference in varietal reaction to this virus. The rolling and mottling are almost masked in Burbank and Irish Cobbler. In some other varieties, such as Earliest-of-all, Idaho Rural, Bliss Triumph and White Rose, the leaves show distinct mottling and rolling; these symptoms are reduced by high temperatures. The disease appears to be transmissible by leaf-mutilation and core-grafting, and McKay and Dykstra claim to have transmitted it very occasionally by the aphides *Macrosiphum gei*, *Myzus pelargonii* and *Myzus persicæ*. There is no information on the pathological histology of this disease. The incubation period of the virus in the plant is about 15–20 days at a warm temperature. Inactivation of the virus sets in after 24–38 hours' ageing *in vitro* and the thermal death-point lies between 70–75° C. As regards dilution, it may be quite infectious at a dilution of 1 : 200. Leaf rolling mosaic seems to be confined to America, though the suggestion has been made that the disease might be due to the same entity as that causing paracrinkle (27).

Yellow Dwarf. The haulms, which are greatly dwarfed, turn greenish-yellow and often die prematurely from the top downwards. The top leaves may be purpled and rolled upward. Cutting reveals brown specks in the stems, especially in the joints. Tubers on affected plants contain brown spots, and are often cracked, even though small (54). Nothing is known of the mode of transmission of the virus. The disease occurs in some eastern states of the U.S.A. (2).

Yellow Top. This disease has been described in America by Folsom (12). It is characterized by dwarfing, by spindliness, sometimes by extreme chlorosis or by distinct rolling, by stiff leaf texture and sometimes by tuber net necrosis, and is tuber-perpetuated. The disease appears to be transmissible by means of grafts.

Psyllid Yellows. An obscure disease of the potato which may be due to a virus and which is connected with the potato psylla (*Paratrioza cockerelli* Sulc.) has been described by Richards (33). The disease has some of the symptoms of both leaf roll and witch's broom. The lower leaves roll upward, turn yellow, red, pink or purple and die prematurely. The joints of the stems often swell, and bear thick shoots, aerial tubers and rosettes of small leaves. According to Binkley (5) the disease is transmitted by *P. cockerelli* after feeding on infected plants ; it is not transmitted by non-viruliferous psyllids. The disease appears to be perpetuated by the tuber, and when mixed with mosaic in the same plant still produces its distinct and specific effect (44). Psyllid yellows has only been recorded from the United States of America, where it occurs in Utah, Montana and California.

Aucuba Mosaic. This disease was first adequately described by Quanjer (30); it is characterized by prominent, localized, yellow patches which are more or less round and regular in outline; there is no leaf distortion. The name " aucuba mosaic " was given because of a supposed similarity to the variegations in the foliage of *Aucuba japonica*, and it should not be confused with " aucuba mosaic " of the tomato, which is due to quite a different virus (see p. 376). It may be well to mention here that symptoms closely resembling aucuba mosaic of potato may arise owing to other causes, in which frost is probably a likely factor. The virus is transmissible by grafting, but the insect vectors are not known. Aucuba mosaic can be differentiated from potato calico, which it somewhat resembles, by the primary symptoms—small round yellow spots in the former and large yellow areas in the latter.

Cytological studies of potato plants affected with aucuba mosaic show that the chlorosis is due to loss of green pigment, coupled with excessive quantities of starch in the plastids and alterations in the structure of the chloroplasts, which sometimes disintegrate. Intracellular inclusions have not been demonstrated (8). Aucuba mosaic is present in Europe and the U.S.A. and the virus causing it is thought to be the same in both continents (27).

Viruses of the Potato Mosaic Group

It cannot be too strongly emphasized that the terms crinkle, mosaic, streak, etc., applied to a potato virus disease are descriptive only of a set of symptoms and do not designate a specific virus or virus complex *unless the potato variety which is affected is also named.* Unless this condition is fulfilled, the disease only is described and little clue is given to the nature of the causal entity or entities. Much of the confusion existing in the literature on the potato mosaic group is because virus workers have not appreciated this fact and have either described the effects of different viruses as " mosaic," for example, or have described the same virus, on different potato varieties, as a number of distinct viruses. Thus it is possible, using the Y virus alone, to produce crinkle, mosaic or leaf-drop streak, according to the potato variety and, as regards the last-named symptom, according to the number of seasons the plant has been infected.

Paracrinkle (Virus " E " of Bawden). This virus was first described in 1930 by Salaman and Le Pelley (37) and was so named to differentiate it from Murphy's crinkle. It was discovered that all plants tested of the variety King Edward " carried " this virus without symptoms, but when grafted on to Arran Victory produced a severe crinkle disease. The variety President also carries this virus without symptoms. Murphy and M'Kay (27) have suggested affinities between paracrinkle and leaf rolling mosaic, but the fact that the latter virus causes symptoms in President seems to disprove this theory. The following are the chief symptoms of paracrinkle on Arran Victory, the first sign is a single bright blotchy patch of chlorosis on the junction of the larger veins, but even at the earliest stage this is accompanied by local puckering and deformity together with an early waving of the leaf edge. The blotchy spots increase in number and become necrotic, bringing severe deformities in their train. Growth of the leaflets is more or less inhibited, and by reason of the waving, deformity and rugosity they appear smaller than their actual area warrants. Necrotic spots are common on the surface of the leaf, and short brown streaks occur on the veins of the under surface, as well as on the petioles and stems. In the season following that of infection, the stems of the diseased plant are numerous, short and brittle, while the leafage is clumped and grossly deformed. Plants derived from diseased tubers of Arran Victory grown in the field present a typical picture of the condition known as " curly dwarf," in which the plant is not more than 6–9 inches high and is of the " rosette " type. Intra-cellular inclusions are not present.

This virus is not sap-transmissible and has been disseminated by grafting only ; the insect vectors are not known. Paracrinkle has so far been described only from England, occurring naturally in the variety King Edward. Salaman (35) considers that paracrinkle is due to a virus complex, but the writer prefers to regard it at the moment as a single virus. In the writer's opinion this virus is restricted in host range to the potato plant and is not transmissible to other members of the Solanaceæ.

Potato Virus X of Kenneth Smith (51) (Simple Mosaic of Murphy and M'Kay (27); Common Mosaic of Quanjer (31); "healthy potato virus" of Johnson (18); tobacco ringspot of Johnson (18) and Kenneth Smith (46); Hy. IV of Hamilton (15); top-necrosis of Quanjer (32)).

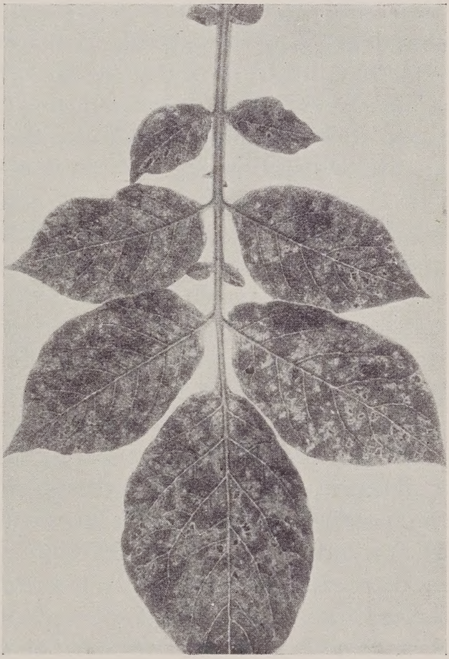

Fig. 48.—Potato virus X on potato, var. President. Note the interveinal character of the mottling.

It is to be hoped that the various mosaic viruses affecting the potato plant will one day be classified and differentiated, and when that is achieved the viruses will be more conveniently lettered or numbered. For this reason the writer has listed the most common potato mosaic virus as virus X, under which designation it has been studied by him upon a large variety of hosts (51). For the same reasons the virus in the succeeding paragraph is dealt with as Y. The symptoms of the X virus upon a selection of potato varieties are given herewith. Out of nineteen varieties examined by Salaman and Bawden (36) three responded with a top-necrosis, the variety King Edward being usually killed by an acute necrosis. As this potato variety seems invariably to be infected with the virus of paracrinkle (see above), it cannot be said what the true reaction of the X virus would be on this potato. Two other varieties, Arran Crest and Epicure, developed an acute necrosis of the growing points in fourteen days, which was usually fatal. The X virus thus produces a necrotic disease (top necrosis) on Epicure, Arran Crest and King Edward, but only a mild mosaic mottling without necrosis on Arran Victory and President (see Fig. 48); on Eclipse it produces a more pronounced mottling, and it is carried without any visible symptoms by Up-to-Date and Di Vernon.

The X virus is transmissible by core-grafts and by inoculation; it is not aphis-transmitted and the insect vectors are not known. In histological studies, it is not easy to distinguish the pale areas in sections of fixed material except by a slight underdevelopment of the plastids. In fresh material the chloroplasts of the pale areas are slightly smaller and not quite so deep a green as those of the green areas (8). X-bodies are usually present and were first described by the writer as occurring in the potato plant, from what was probably this virus disease (45) (see Fig. 48).

In those varieties where a necrotic disease (top necrosis) develops, internal symptoms are produced in the petioles, stems and tubers, and consist of necrotic changes which originate in the phloem, and spread from thence to all other tissues (3). The following are some of the physical properties of the X virus as determined by the present writer. The virus is filterable through an L_3 Pasteur-Chamberland candle and occasionally through an L_5; it passes a collodion membrane of A.P.S. $0{\cdot}425\ \mu$ but not one of $0{\cdot}35\ \mu$, and from this a particle size of about $0{\cdot}20\ \mu$ is calculated. Its longevity *in vitro* in a sterile L_3 filtrate at room temperature is, so far as tested, six weeks, while at a temperature of $-10°$ C. it persists for eighty-six days or longer. It is tolerant of glycerine and toluol and withstands a dilution of $1/10^4$, its thermal death-point is 66° C. The X virus appears to be present wherever the potato is grown; it is found in all commercial stocks of American potato varieties and it is the commonest virus in European potatoes. The writer has also isolated it from potatoes sent from Tasmania and Rhodesia. It occurs naturally in other Solanaceous plants and has been re-described as causing a new virus disease of Hyoscyamus by Hamilton (15) under the designation Hy. IV.

Interveinal Mosaic. (= Duke of York Mosaic of Quanjer (30); Super-mild Mosaic of Young and Morris (54).)

On variety Duke of York, pale patches develop, chiefly between the veins; the leaf tissues directly in contact with the veins remain green. Margins of leaflets are often a little undulated and turned upwards (31). The virus appears to be transmissible by core-grafting. There is no exact information on the insect vectors. Histological examination of the disease shows that the chlorotic areas of the leaves are thinner than the green areas, due to the inhibited development of the cells in the pale areas. Intracellular inclusions, or X-bodies, are abundant in plants suffering from prolonged effects of interveinal mosaic (8). The disease occurs in Europe and the U.S.A., and is considered by Murphy and M'Kay (27) to be due to the same virus in both continents. There is a certain amount of evidence that the potato virus X can also produce an "interveinal mosaic" particularly on President, and it may well be that the writer's X virus and that causing "interveinal mosaic" are the same entities.

Potato Virus Y of Kenneth Smith (51). (Streak of Orton (28), Leaf-drop Streak of Murphy (23), Stipple-Streak of Atanasoff (1), Acropetal necrosis of Quanjer (32), Hy. II. of Hamilton (15), Veinbanding virus of Valleau and Johnson (52)).

This virus is one of the commonest and most destructive of potato viruses in England. The chief symptom picture is that of "leaf-drop streak," which it produces in the current season on the varieties President, Up-to-Date, Majestic, Arran Consul, King George and Duke of York, and this will be described. After an incubation period of fifteen to twenty days a blotchy mottle appears, spreading from the veins and affecting the uppermost leaves only. This mottle later becomes intensified and is accompanied by some wrinkling and waving of the leaves. A little later necrosis appears on the undersides of the veins of leaves occupying an intermediate position on the stem, the upper surface of which may for a time look normal (see Fig. 49). These necroses increase in severity and spread most rapidly along the course of the veins on the under-surface of the leaf, appearing as elongated brown stripes on the petioles and as blotches between veins; later they penetrate the leaf tissue and become evident on the upper surface. The necroses pass down the petiole to the main stem and the leaf then collapses, rapidly withers and remains hanging, as it were, by a thread (see Fig. 50). The stem

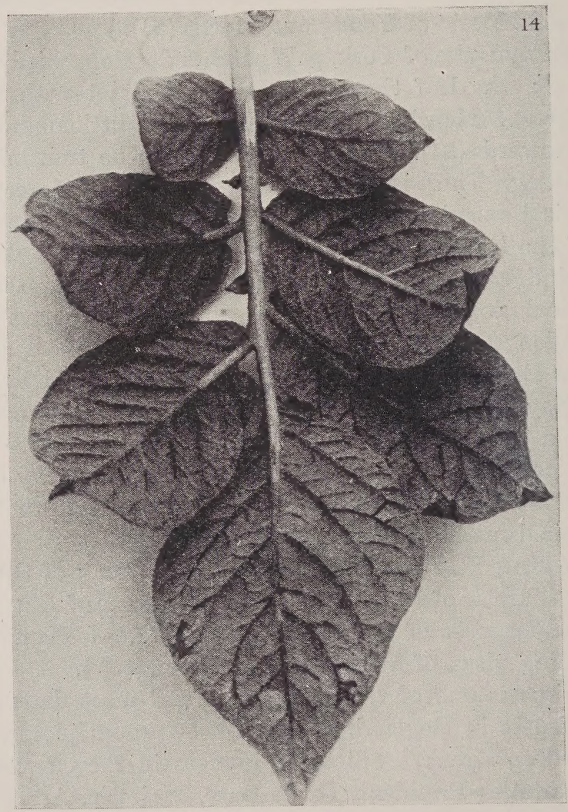

Fig. 49.—Potato virus Y on potato, var. President. Primary symptoms in the form of veinal lesions on the undersides of the leaves.

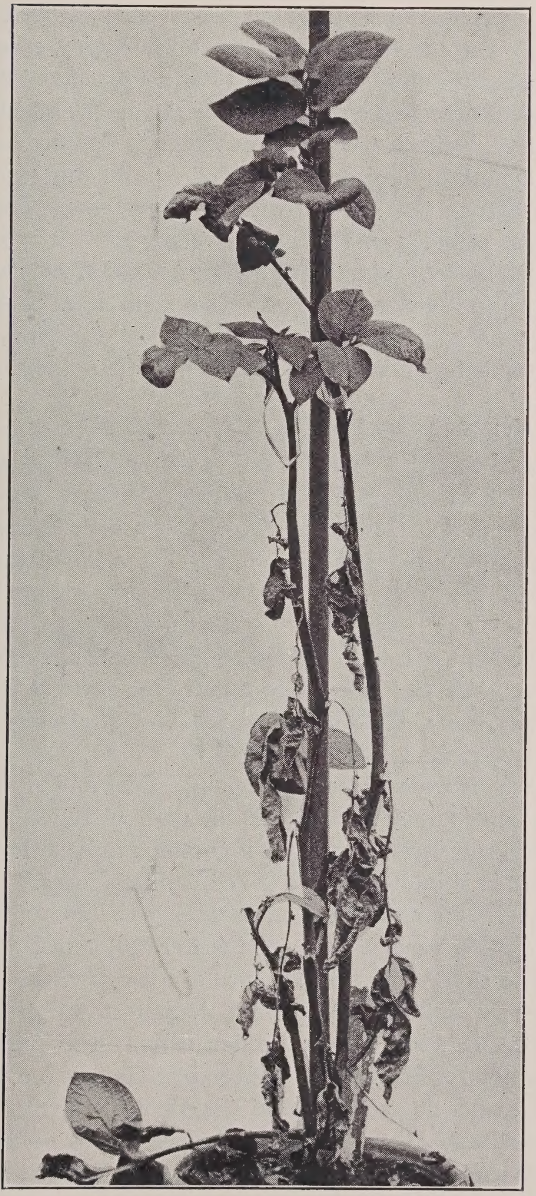

Fig. 50.—Potato virus Y on potato, var. President. Systemic infection showing leaf-drop-streak.

itself frequently exhibits elongated brown stripes. The topmost leaves rarely become necrotic but are often highly crinkled (36). In the field, potato plants so diseased frequently have the hanging necrotic leaves detached by the wind, so giving the affected plants a " palm tree " effect. On Arran Victory, Epicure and Arran Chief the symptoms are only those of a mild mosaic mottling. Di Vernon carries the Y virus without symptoms. The Y virus is sap-transmissible and the insect vector is the aphis *Myzus persicæ* Sulz., but not, apparently, other species of potato aphides.

Internal symptoms are seen in the stem and petioles, and consist of a necrosis affecting chiefly the collenchyma, the vascular tissues being normal (3). X-bodies do not occur in this disease.

The following physical properties of the Y virus have been determined by the writer ; it is easily adsorbed to Kieselguhr and, probably for this reason, will not pass an L_3 Pasteur-Chamberland, or Berkfeld, candle. It will, however, pass a collodion membrane of average pore diameter of 0·425 μ, and has a calculated particle size of 0·25 μ. Its longevity *in vitro* at room temperature is twenty-four to forty-eight hours, but it is very sensitive to temperature and may be kept viable in extracted juice for thirty-seven days or longer at —10° C. It is more rapidly inactivated by dilution than the X virus, and its dilution end-point is about $1 : 10^3$, its thermal death-point is 52° C.

The Y virus is widespread in England, and probably in Europe generally ; it occurs in America and is perhaps the same virus as that causing American streak. It is common in American tobacco fields, where it is known as the " Veinbanding virus."

Virus Z. In a statement demonstrating the composite nature of certain potato virus diseases the writer (50) showed that there was evidence suggesting the presence of a third virus, other than the entities X and Y. In 1932 Salaman stated that he had discovered a third virus element, which he named Z. The following are the characters of this virus as given by Salaman (35) : it is non-inoculable, causes no symptoms on President, Di Vernon, Epicure, King Edward and Up-to-Date and no symptoms on *Datura* and tobacco. On Arran Victory, by grafting only, it is said to produce a veinal mottle accompanied by a slight ruffling of the leaf surface, but no waving, deformity or necroses.

Virus A. Murphy and M'Kay (26) have recently described another virus, which they have also named alphabetically—in this case A. This virus occurs in the potato variety Irish Chieftain, and is considered by Murphy and M'Kay to be a necessary constituent of the potato virus disease " crinkle." This question is further dealt with in the discussion on virus complexes. The following are some of the characteristics of the A virus. On Irish Chieftain few or no symptoms are produced ; a faint mosaic mottle

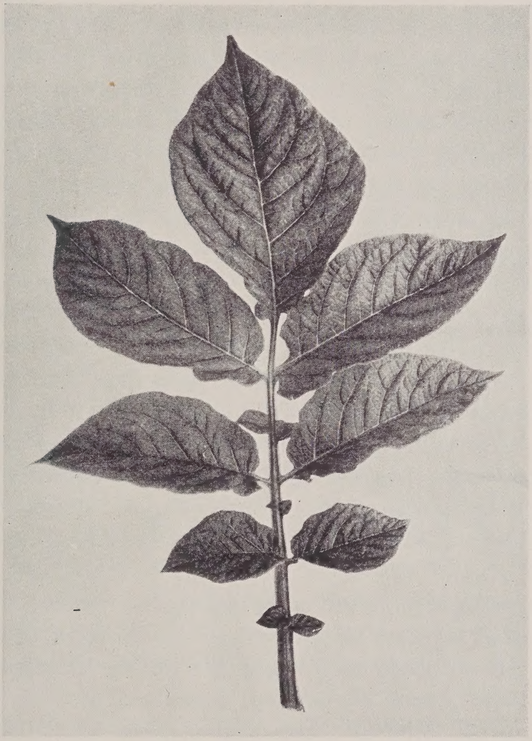

Fig. 51.—Potato virus A on potato, var. President, showing a slight general chlorosis or mottle. (After Murphy and M'Kay.)

accompanied by a slight marginal undulation is frequently seen in the early stages of growth under glass. On the variety President, the symptoms are a faint mosaic (see Fig. 51). Following infection, a slight general chlorosis appears on the upper portion of the plant, and a yellowish mottling can be seen which consists of partially green areas interspersed with normally green ones. Arran Victory has been infected twice with this virus, and in each case a slight mosaic followed, either in the same season or the following one. When inoculated (= grafted ?) to Up-to-Date " carrying " simple mosaic (= X) a severe and often fatal necrotic disease ensued. The symptoms are those usually associated with streak, consisting of black-spotting of the lamina of the leaf, streaking of the veins, petioles and stems, leaf-drop, dwarfing and premature death. Necrosis appears also in some of the tubers, sometimes with fatal effects. Virus A is apparently not sap-transmissible and produces no symptoms upon, and cannot be recovered from, *Datura stramonium*. The insect vector is said to be *Myzus persicæ* Sulz. (21). The fact that the A virus produces recognizable symptoms in President appears to differentiate it from Salaman's Z virus. According to Loughnane (21), the A virus is transmissible to tobacco, on which it produces veinbanding symptoms.

Streak Viruses (other than Virus Y). Virus B of Bawden. (=Up-to-Date Streak of Murphy, Top Necrosis Type A (1) of Quanjer).

This virus seems almost universal in the variety Up-to-Date, and is carried without symptoms ; it is usually accompanied by virus X. It appears to be non-inoculable, because grafts of Up-to-Date to Arran Victory and President produced a necrotic disease (top necrosis) in these varieties, whereas sap-inoculation from the same Up-to-Date to the same varieties produced no such effect. Needle inoculation to tobacco and *Datura stramonium* is followed in these plants by symptoms typical of virus X. Return inoculations from such infected tobaccos to Arran Victory and President do not produce top necrosis (4). Virus B, when transmitted by grafting, produces top necrosis in the following varieties : Arran Victory, President, Arran Chief, Arran Comrade, Kerr's Pink and Sharpe's Express. In addition to Up-to-Date the following varieties " carry " virus B without symptoms: Arran Banner, Arran Consul, Duke of York, Eclipse, Great Scot, Green Mountain, Majestic, Di Vernon, King George, Champion and Rhoderick Dhu. According to Quanjer (32) what is apparently the same virus is semi-latent in the Dutch variety Zeeland Blue, in which it causes a mosaic. The viruses B and X appear to be almost universally present in American potato varieties other than seedlings. Virus B appears to be transmissible only by grafting; it is doubtful if it is communicable to any hosts other than the potato plant. The insect vectors are not known.

Streak Viruses (other than Virus Y) (*continued*).

Virus C. This virus has been described by Bawden (4) as occurring in the variety Di Vernon and has been found only in this variety. When scions from these Di Vernon plants are grafted to virus-free Arran Victory and President plants, top necrosis is produced in the latter, whilst the former reacts with mosaic symptoms only. Grafts from such mosaic Arran Victory plants to President again give top necrosis. This non-production of top necrosis in Arran Victory suffices to differentiate this virus from virus B. Additional evidence, moreover, of the specific nature of the virus B is obtained by transmitting it by grafting to the varieties Arran Banner, Arran Consul, Duke of York, Eclipse, Majestic and Up-to-Date, which carry virus B without symptoms, but all of which develop top necrosis when grafted with virus C. This virus apparently is not sap-transmissible, and the host range seems to be restricted to the potato plant. Information on the mode of dissemination of virus C is lacking.

Virus D (Foliar necrosis). This streak virus has been described by Bawden (4) from variety President. He states that it differs from other potato viruses in that it produces different necrotic diseases in various varieties. In Up-to-Date, Epicure, Abundance and Majestic it produces top necrosis, whilst infected President, Arran Victory, Arran Chief, Kerr's Pink, Golden Wonder, British Queen and other varieties show foliar necrosis. Di Vernon and Great Scot carry virus D without symptoms.

The first symptom of foliar necrosis appears about fourteen days after infection and consists of necrotic blotches, which are most prominent on the under-surface of the intermediate leaves. These increase in size and spread over the whole area of the leaf, which quickly wilts and falls. The main growing point and side-buds are unaffected. These primary symptoms are then followed by a secondary stage in which the upper leaves show isolated interveinal necroses with some mottle and leaf deformity. The necroses at this stage differ from those first produced in being black, hard and brittle, and do not spread over the whole leaf. Tubers from infected plants show no pathological signs. Plants grown from these tubers show symptoms corresponding to the secondary stage of the disease in the year of infection. The plants are smaller than normal, their leaves are mottled and deformed showing isolated necroses, but there is no wilting or falling of the leaf. Virus D is " carried " without symptoms by *Datura stramonium.*

Potato Virus Complexes. Recent work of the writer (51) has demonstrated the composite nature of certain of the potato mosaic diseases by isolating from them two virus entities which, as already described, have been designated as X and Y. He also stated that there probably existed a third virus in crinkle and streak. Valleau and Johnson (52) and Koch (19) have also demonstrated the dual nature of the American potato virus disease known as rugose mosaic, while Murphy and M'Kay (26) have recently shown that European crinkle is composed of two viruses.

Crinkle of Murphy (Crinkle " A " of Salaman : probably the American mild mosaic). The writer showed (50) that the potato crinkle studied by him contained two viruses, X and Y, with evidence of a third constituent. Murphy (24), while admitting this, states that crinkle consists of X and a third constituent which he has named virus A, but holds that virus Y, when present, is in the nature of a contaminant or is incidental, and its presence is in no way essential to the production of true crinkle. Here again the misunderstanding is partly due to the fact that the writer referred to a set of crinkle-like symptoms, while Murphy had in mind a specific disease. The following are the symptoms of crinkle, viruses A + X (X = simple mosaic) as described by Murphy (23): affected plants are typically bushy, dwarfed specimens of about the same size and form as the low-headed type of leaf roll. The compact appearance of curly dwarf is absent. The colour is a pale green, but this feature is not marked. The most characteristic symptom is a pronounced puckering and downward curving of the leaves. There is no distinct spotting as in mosaic, but diffused, slightly yellowish areas occur, all over the foliage (see Fig. 52). As death approaches, this colour becomes more pronounced and is accompanied by rusty-brown spots, beginning near the tips of the leaves. The foliage is brittle and easily injured.

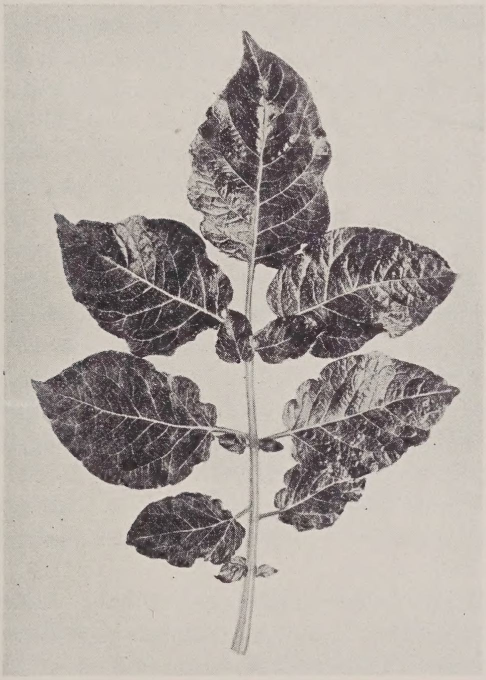

Fig. 52.—Crinkle on potato, var. Irish Chieftain. This disease is caused by a mixture of the two viruses, A and X. (After Murphy and M'Kay.)

The first symptom of crinkle on variety President is the development of a pale patch in the apical leaves, further pale patches appear, and contemporary with their formation there occurs a shrinkage of the tissues involved and a consequent degree of deformity. Accompanying the mottling and deformity there is a definite waving of the edge of the leaflets. Occasionally minute necroses resulting in black spots may develop, and there may also form fine necrotic streaks along the veins on the underside of the leaflets. On Arran Victory the crinkle symptoms are less severe than on the foregoing. The main difference in the symptom picture on the two varieties lies in the fact that in Arran Victory, whilst necroses and deformity are rare and less severe, mottling may be very distinct, and its formation as chlorotic patches extending from the cleared veins to the angles between them is characteristic. In Arran Victory plants, long affected with crinkle, the mottling becomes faint and diffuse while the leaf surface is glazed with an intensification of the green colour (34). On variety Up-to-Date crinkle takes the form of a severe and usually fatal disease. Following infection, dark necrotic spots appear on the leaves, with streaking of the veins and petioles. The leaves are sometimes killed and remain hanging, or the upper portion of the shoot may be killed. If tubers are produced there are lesions on their surfaces and the plants arising from them, if any, are usually necrotic dwarfs (26).

It is not known how the disease crinkle is transmitted in the field, since the constituent virus X (=simple mosaic) is apparently not insect-borne.

Rugose Mosaic (American disease: probably a combination of the European viruses, X and Y). It has been demonstrated by Koch (19) and Valleau and Johnson (52) that the American disease known as rugose mosaic, which somewhat resembles the European disease crinkle, is composite in nature. It is probable that the crinkle disease in potato Myatt's Ashleaf investigated by the writer (51) and which contained the two viruses X and Y was a similar disease to the American rugose mosaic. The following are the symptoms of rugose mosaic on Green Mountain (40): distinct dwarfing, chlorosis and diffused mottling, a rugose type of wrinkling, and a tendency to show brittleness, spotting, streaking, leaf dropping and premature death.

The following are the symptoms of rugose mosaic as given by McKay and Dykstra (22), presumably upon the varieties Burbank, Bliss Triumph and Earliest-of-all. They state that the disease can be readily differentiated from mild and crinkle mosaic, in that the mottled areas are smaller, more numerous and grouped closer to the main veins. During high temperatures the mottling may be completely masked, but the crinkling of the leaves and their tendency to curl slightly downwards persist and make the identification of this disease certain. Some of the veins of the lower leaves are generally necrotic, resembling black pencil-like lines. The affected plants are noticeably stunted and generally die prematurely.

The necrotic streaking of the veins and the leaf-drop-streak, which is often a current season symptom of rugose mosaic, are due to the presence of the virus Y, a constituent of this disease, and are characteristic of this virus. The disease is produced in the field by aphis transmission of virus Y, which is carried by *Myzus persicæ*, this virus added to the " healthy potato virus," otherwise X, universally present in American potato stocks, produces the rugose mosaic disease. It seems probable, therefore, that the disease is not transmitted *as such* by insects but arises from the introduction of the Y virus to a potato plant already " carrying " the X virus. The disease is transmissible by needle inoculation, since both constituent viruses are sap-inoculable.

Crinkle Mosaic. (American disease : possibly Murphy's crinkle + another virus). Symptoms on Green Mountain resemble those of American mild mosaic (= European crinkle), but the disease is characterized by a much greater degree of crinkling, *i.e.*, of wrinkling, waviness of the margin and ruffling (41). On variety Earliest-of-all the disease is characterized by a prominent mottling and crinkling of the leaflets. The mottled spots are large and have a tendency to be noticeable, especially around the margin of the leaflets. It can be distinguished from rugose mosaic by the larger blotches and by the absence of necrosis on the veins of the lower leaves (22). The large size of the mottled spots appears to be a characteristic of this disease. Murphy and M'Kay (27) state of this disorder: "No European disease is known which corresponds to crinkle mosaic, and it is probably a mixture of crinkle with another virus or viruses." McKay and Dykstra (22) state that they transmitted the disease by leaf mutilation but with a low percentage of infection. One case of transmission by *Myzus persicæ* and one by *Myzus circumflexus* on tuber sprouts were also recorded by these two workers.

From a survey of the foregoing account of the virus diseases of the potato, two facts are evident—the first is the extreme frequency with which the various viruses must become mixed in the potato plant and the consequent rare occurrence of a potato plant affected with a single virus, and the second fact is the lack of knowledge as to the means of dissemination of the various viruses in the field. Apart from the transmission of leaf roll by *Myzus persicæ* Sulz. and one or two allied aphis species, the only certain information on the insect dissemination of the potato viruses seems to be that of virus Y and the aphis *Myzus persicæ*, to which must now be added a recent statement on the transmission of virus A by the same insect (21). The claims of various virus workers that *Myzus persicæ* has transmitted mosaic, crinkle and streak can all be explained on the basis of the transfer of virus Y to different potato varieties or potato plants already infected with the X virus. All attempts on the part of the present writer to transmit virus X or paracrinkle by means of aphides and other insects have failed, and in the same way nothing appears to be known of the vectors of the viruses B, C, etc.

In considering the possibility of differentiating and classifying the various potato mosaic viruses, the first thing that strikes one is the difficulty of separating out the various entities. The second point is, having separated them, how to study any characteristics they may have, other than symptoms, since several of those viruses appear not to be sap-inoculable. In attempting to study the potato virus instead of the disease, the writer has isolated and named two separate virus entities (51) by means of differential hosts and by the selective power of transmission shown by the insect vector. This technique, however, cannot be applied to all the viruses, some of which appear to be confined to the potato plant as a host and are apparently only transmissible by grafting. For the present, therefore, those particular viruses can only be studied by their symptoms, and since the potato plant appears to be the only host they must be studied on that plant. Bawden (4) suggests that by inoculating and grafting virus-diseased potatoes to a suitably chosen series of differential potato hosts and by distinguishing on those hosts between the necrotic symptoms produced, it should be possible to identify all the commonly occurring potato viruses of the mosaic group. The following potato varieties are thought to suffice at the moment as differential hosts : Up-to-Date, President, Arran Victory and Epicure. If on needle inoculation to any one variety, a different result is obtained from that produced by grafting, then the existence of more than one virus should be suspected. Acropetal necrosis on President and Up-to-Date suffices to show the presence of virus Y ; foliar necrosis on President and Arran Victory indicates virus D ; top necrosis on Up-to-Date, President and Epicure shows virus C ; and the same symptoms on President, Arran Victory and Epicure show virus B ; top necrosis on Epicure only, indicates virus X, and on Up-to-Date only, virus A. The necrotic effects of viruses A, B and C are produced by grafting only.

Bawden has compiled the following table which sets out the necrotic reaction produced by the various viruses on the differential potato hosts. A plus sign under the heading " inoculability " indicates that the virus is transmitted by both grafting and needle inoculation, a minus sign alone by grafting only. A minus sign under any variety indicates that no necrotic symptoms are produced by the corresponding virus.

For the identification of viruses A, B, C and E, which are only graft-transmissible, some such method as that indicated by Bawden must be used, as obviously it is not possible to study the physical properties of such viruses nor, with the possible exception of virus A, since they are apparently confined in host range to the potato plant, can they be examined by the method of differential hosts. For the identification of the X and Y viruses the present writer's technique of inoculation to differential hosts (51) may be used, and in addition their physical properties, which differ markedly, may be examined.

Virus.	Inoculability.	Up-to-Date.	Epicure.	President.	Arran Victory.
A	—	top necrosis	—	—	—
B	—	—	top necrosis	top necrosis	top necrosis
C	—	top necrosis	top necrosis	top necrosis	—
D	+	top necrosis	top necrosis	foliar necrosis	foliar necrosis
E	—	—	—	—	acute crinkle
X	+	—	top necrosis	—	—
Y	+	acropetal necrosis	—	acropetal necrosis	—

Certain viruses, which are capable of affecting the potato plant under experimental conditions and which may occur rarely on this plant under natural conditions, are briefly touched upon. They are more fully described in Chapters XIII. and XIV.

The potato plant is susceptible to the virus of curly top of the sugar beet, plants so affected are stunted with yellowish, inward-rolled leaflets and bent petioles (43). The writer has found the potato fairly susceptible to infection with the virus of tomato spotted wilt (see p. 377) both by inoculation and by means of the insect vector *Thrips tabaci*. Symptoms take the form of ring-like necrotic spots followed by a general necrosis (48). A disease which may be due to the same virus has been recently described as occurring in the potato British Queen, near Eureka, California (42). A necrosis called " British Queen Streak " is manifested by fine necrotic streaking of leaf veins, slight streaking of the stem and small necrotic lesions.

Most of the viruses of the tobacco mosaic type produce local lesions only upon potato plants, but the virus may become systemic, depending upon the variety of potato (6). The virus of a tobacco ringspot produces local symptoms in potato, but does not apparently become systemic (16). It is perhaps worth recording that the virus of aster yellows, which appears to have the widest host range of all plant viruses, does not attack the potato plant (20).

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CHAPTER XIII

THE VIRUS DISEASES OF PLANTS

A descriptive account of the virus diseases of plants, other than the potato, classified according to their hosts.

In this and the succeeding chapter a description is given of the chief virus diseases other than those of the potato plant, classified according to the plant hosts. It is not possible in the space available to give a very full account of each disease, but an effort has been made to give the essential facts, and attention is paid, as in the case of potato virus diseases, to symptoms, modes of transmission, identity of insect vectors, the pathological histology and presence of intracellular inclusions, any physically and chemically determinable properties and geographical distribution.

Dicotyledons

Ranunculaceæ. An apparent virus disease of *Anemone nemorosa* has been described (26). This disease is known as "alloiophylly," and is characterized by a strikingly spreading habit of the affected plants. The leaves are frequently much thickened and misshapen, with abnormally shallow indentations. The stem or leaf stalks are almost always more or less noticeably thickened. The flowers are mostly suppressed, those that occasionally develop being deformed. Certain inclusion bodies have been described in the living phloem cells ; these vary in shape and may be thread-like in form or oval and elongate. Rhizomes of diseased anemones and healthy rhizomes grown in proximity to infected plants appear to produce diseased progeny the following year. The virus appears to be communicated to healthy plants by placing parts of infected anemones in the soil or by mixing the soil in which diseased plants have grown with that of healthy plants. The virus is transmitted by smearing the buds of healthy plants with the sap of infected ones; the insect vectors are not known. The disease has been described from Germany and, according to Klebahn's work, is one of the few viruses that are soil-transmitted.

A virus disease of the garden varieties of perennial delphinium has been described (51A). The symptoms are very similar to those of one of the tobacco ringspot viruses, though apparently they are due to a separate entity. There are chlorotic ring patterns on individual lobes and sometimes patterns which extend into each of the lobes of the leaf, following more or less closely the primary veins. The patterns may or may not be present as the plants start growth in the spring, but are likely to become more prominent as the leaves become older. After flowering, a few or all of the leaves may bleach to a light greenish yellow similar to the bleaching of yellow ringspot of tobacco.

The virus is sap-transmissible by rubbing to tobacco, on which it produces necrotic ring and line patterns. The cucumber plant is also susceptible to this virus, affected plants showing a conspicuous mosaic disease.

Plants in three families are apparently susceptible to the delphinium virus, *i.e.*, Ranunculaceæ, Solanaceæ and Cucurbitaceæ. The insect vectors are not known.

The disease has been recorded from Lexington, Kentucky and from the Yonkers neighbourhood, New York.

Cruciferæ. A mosaic virus affects many species of Cruciferæ and the symptoms are described as they appear on certain varieties of Brassicæ (8). On Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* Zenker), plants occasionally show the usual type of mosaic mottling, but the more common symptom is the development of round yellow blotches scattered evenly over the leaves but not raised. Later the tissues round the edges of the yellow areas die, producing an effect of ringspotting. In the cauliflower, symptoms are somewhat similar to the foregoing with yellow spots very slightly raised on a normal-appearing green leaf. Tissues round the edge of the cauliflower leaf do not die as in the case of affected Brussels sprouts, and there is no leaf distortion or necrosis. In affected swedes (rutabagas) the leaves show yellowing, ruffling and are sometimes blistered. The following cruciferous plants have been described as susceptible to a mosaic virus which, in the absence of evidence to the contrary, is considered to be the same infective agent, turnip (14), mustard, Chinese cabbage (*Brassica chinensis* L.) (44), *Raphanus* and *Sinapis arvensis* (16). The cabbage plant appears to be highly resistant to the virus. Symptoms vary widely with the different host plants and with environmental conditions. At 70°–80° F. the mustard and Chinese cabbage show streak, while at 55°–65° F. even the more susceptible hosts made fair development (8). The virus appears to be sap-transmissible to certain hosts, and the chief insect vector is the cabbage aphis, *Brevicoryne brassicæ* L. *Myzus persicæ* Sulz. may also be concerned in the transmission of this virus. The disease appears to be widely spread throughout Europe and the U.S.A.

Violaceæ (Cultivated Violet). The writer has received from Mr. Hodson, of Reading University, specimens of the cultivated violet, variety Princess of Wales, affected with a typical mosaic disease. The leaves show a strong mosaic mottling of green and yellow with deep indentations, which give the leaf a crinkled appearance. The flowers show marked symptoms of " breaking " (see tulips, p. 393), with white splashes or stripes alternating with the normal colour.

Phytolaccaceæ (*Phytolacca decandra*, L. Pokeweed). Allard (1) describes a mosaic disease of this plant which in all phases of its expression bears a close similarity to tobacco mosaic, but the disease appears to be due to a different virus, as the mosaic of pokeweed is not transmissible to tobacco, nor is the mosaic of tobacco transmissible to pokeweed. Initial symptoms appear in the young leaves, which later may become beautifully mottled and are occasionally distorted as in tobacco mosaic. There is no evidence of soil or seed transmission. The virus is easily transmissible from diseased to healthy pokeweed plants. There is no information on the insect vectors, though Allard suggests a large species of aphis (*Macrosiphum* sp.) as possibly concerned in the spread of this virus.

Malvaceæ (Cotton). The disease of cotton known as " leaf curl " was originally described from Nigeria by Farquharson (13), and later, in 1926, by Jones and Mason (23). Its first recognition as a virus disease was made simultaneously by Golding (15), in Nigeria, and by Kirkpatrick, who named it " leaf crinkle," in the Sudan (24, 25). Symptoms of leaf curl upon Sakellaridis cotton are as follows : a leaf that is fully grown never contracts curl, but a partly grown leaf may develop a thickening of the lower surface of the smaller veins (see Fig. 53). Such thickening starts at a number of points, which gradually tend to join up, until all the veins are affected, sometimes only part of the leaf is affected. Frequently symptoms first appear on the youngest leaves, the older or partly

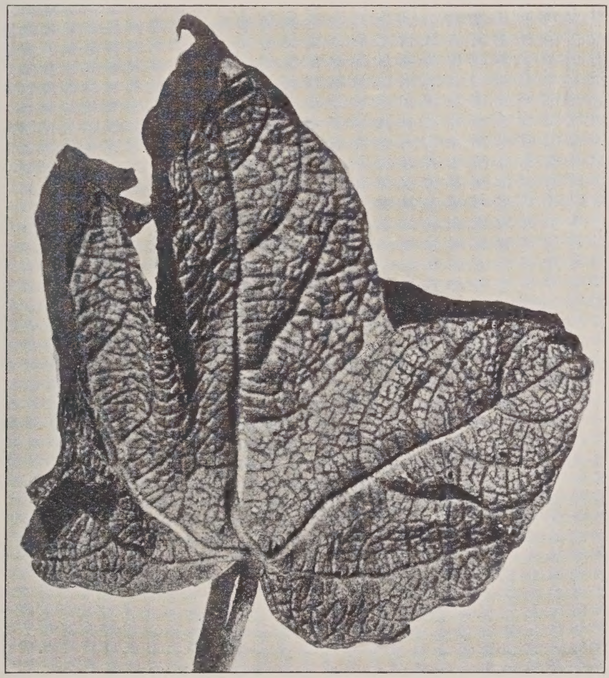

FIG. 53.—Leaf curl of cotton. Sakellaridis cotton leaf with moderately severe symptoms. (After Kirkpatrick.)

grown ones developing normally. If an affected leaf be viewed from beneath against the light, it will be seen that the thickened veins are darker green than the rest of the leaf and consequently opaque instead of translucent, as are the veins of the normal leaf. This character is valuable in diagnosing the disease when present in a mild form with absence of crinkling. In some plants, at a variable interval after the first infection was visible, the symptoms become greatly intensified. The new leaves are small, crinkly and curled at the edges, either downward or upward. Not infrequently oval cup-like foliar outgrowths are produced on the greatly thickened lower sides of the primary veins (cf. tobacco leaf curl, p. 372). The primary stem of the plant often tends to grow taller than normal, the internodes being elongated and irregularly curved, but sometimes the whole plant is stunted in growth. Affected plants are brittle and easily broken (24). The cotton leaf curl virus is transmissible from Sakel cotton to Bamia (*Hibiscus esculentus*), but it is very difficult, if not impossible, to transfer the virus from Bamia to Sakel cotton. Til (*Hibiscus cannabinus*) is susceptible and the symptoms on this plant are very conspicuous, the veins being extremely thick and gnarled. Other host plants appear to be hollyhocks (*Althœa rosea*) and Karkade (*Hisbiscus sabdariffa*). The tomato plant (*Lycopersicum esculentum*) and the zinnia are suspected to be hosts for this virus.

The virus of cotton leaf curl is not sap-transmissible and the insect vector is the whitefly, *Bemisia gossypiperda.* It is not transmitted by the seed nor through the soil (25). Cotton leaf curl has been recorded from the Sudan and from Nigeria, and Mathur (29) states that it has been seen in India on zinnias. It is possible that the disease seen by Mathur may be due to the same virus as that causing tobacco leaf curl (see p. 372), which is also transmissible to zinnias (51). Whether the two diseases are caused by the same infectious entity has not been determined, but they are probably distinct.

Geraniaceæ (*Pelargonium zonale*). The cultivated pelargonium is subject to a suspected virus disease of the mosaic type, which seems to be widely spread in Europe. The first symptom is the appearance on the young leaves of small round or angular yellow spots about 2 mm. or more in diameter, and often densely crowded. These spots may develop into rounded or more or less stellate, or even dendritic, blotches of a bright yellow colour. Later, necrosis of the spots sets in and the tissue dries up and becomes brown. Growth in these areas ceases, but continues in the surrounding

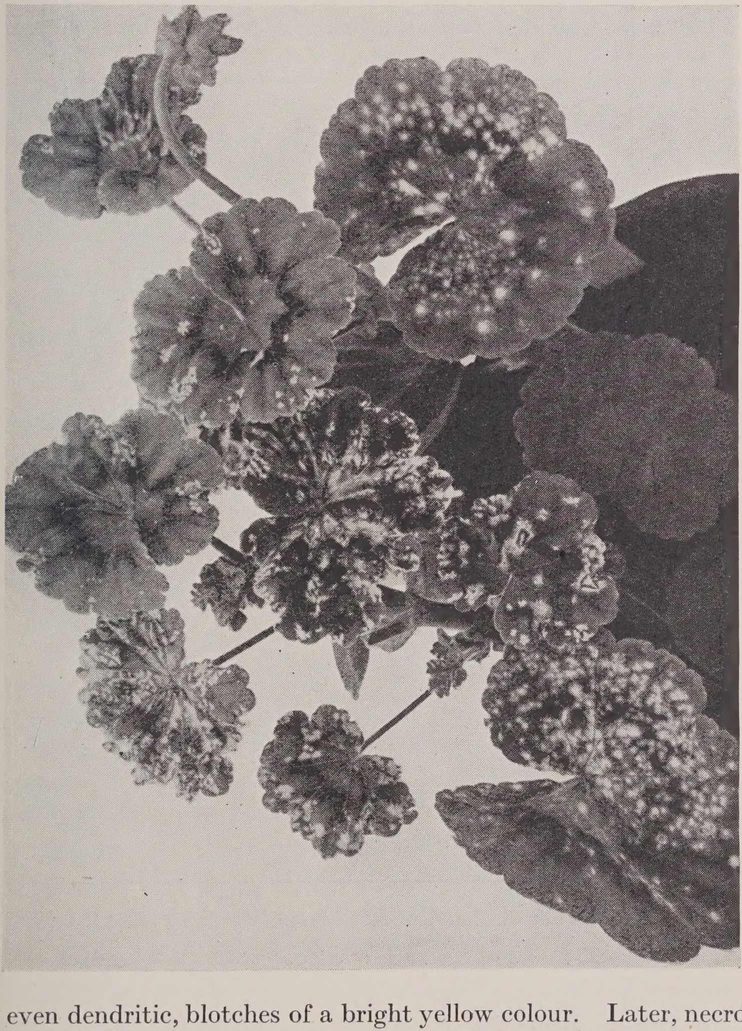

FIG. 54.—Symptoms of a virus disease on the cultivated zonal pelargonium. (After Pethybridge and Smith.)

tissues of the lamina, with the result that the leaf develops a crinkled and puckered aspect, sometimes accompanied by splitting. In severe cases the whole plant becomes malformed and degenerate (see Fig. 54). The virus is transmissible by grafting, but the insect vectors are not known. There is no evidence of seed transmission of the virus. Apparent recovery, *i.e.*, disappearance of the symptoms, is a common occurrence in this disease. Cuttings from such " recovered " plants, however, usually show the symptoms

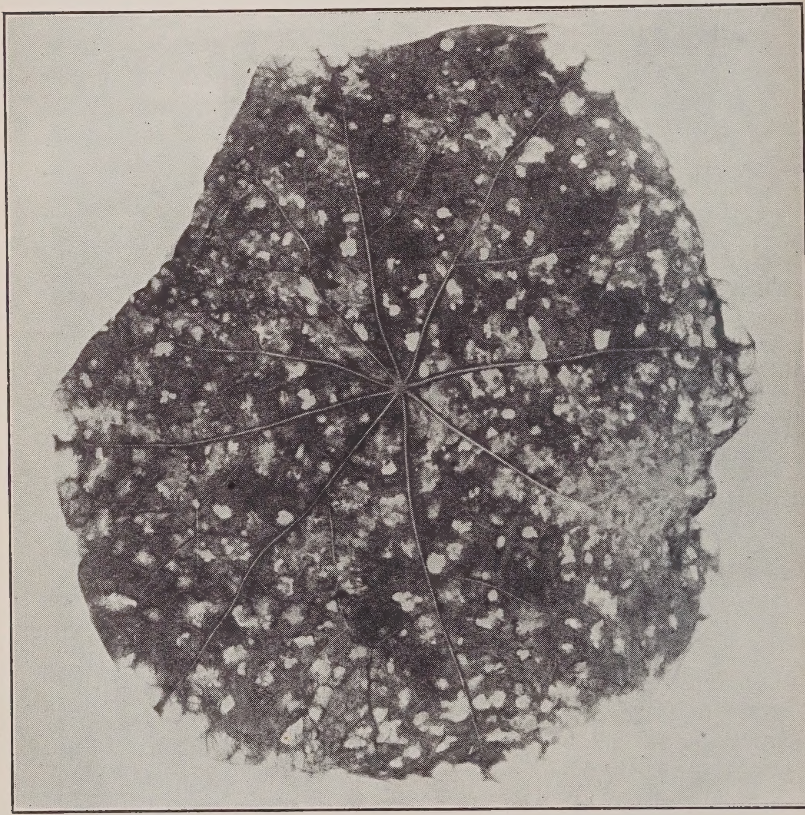

Fig. 55.—Leaf of the double nasturtium (Tropæolaceæ) affected with the virus of tomato spotted wilt.

again the following year. Certain varieties, such as Paul Crampel, appear to be unusually susceptible to the virus, while others, such as the salmon-pink varieties, are more resistant (36).

Celastraceæ (*Euonymus* spp., Spindle tree). An infectious chlorosis of *Euonymus japonicus* and *E. radicans* has been described (43); the disease is characterized by pale stripes along the veins of affected leaves. The virus is transmissible by grafting, but not, apparently, by any other means.

Rhamnaceæ. A tropical species of this group, *Zizyphus* sp., is affected by the so-called "spike" disease, which is probably due to a virus (see Santalaceæ, p. 385).

Vitaceæ (*Vitis vinifera*, the vine). A mosaic disease of the vine has been described by Smolák (48) and in greater detail by Stranak (50). Symptoms take the form of yellowish, indistinctly delimited spots of irregular shape, giving a mottled aspect; later these spots increase in size and become whitish. According to Stranak the secondary and larger veins, and often the parenchymatous tissue, take on a yellowish, whitish or pale green tint. The chief characteristic of vine mosaic seems to lie in the discoloration of the veins. Among the various forms of discoloration caused by the disease it is possible to distinguish the following: the veins alone yellow in the surrounding tissue, the veins and a varying amount of the surrounding tissue discoloured, leaf discoloured in varying degrees, or the leaves entirely of a yellowish colour, later becoming almost white. Stranak states that the chloroplasts are attacked and destroyed in a characteristic manner (cf. Sheffield, p. 38) and the products of disintegration fill the conducting vessels. Intracellular inclusions, deeply staining with Giemsa, are described as the causal organisms of the vine mosaic. The virus appears to be sap-transmissible, by root inoculation and by means of the pruning-knife; there is no soil transmission. The insect vectors are said to be *Lecanium corni* and "aphides" unspecified. The disease is widespread in Czechoslovakia, where it has been known for more than forty years.

A suspected virus disease of the vine must be mentioned; this is leaf curl or "roncet," and is probably the same affection as that known in France as "court noué" (short node), and in South

Africa as "tandpynziekte." The shortening of the internodes is a characteristic symptom and the leaves are indentated, small and crumpled. Petri (37) has described intracellular formations which produce cordons traversing the cells. Little seems to be known of the methods of transmission of this disorder. The disease is serious in France, and is widely spread in the northern hemisphere; it also occurs in South Africa.

Leguminosæ

The various species of clover, garden peas, sweet peas and beans are all susceptible to a mosaic disease. It is probable that more than one virus is concerned in the mosaic diseases of the different species, and this question is further discussed in the description of the disease on different Leguminous plants.

Clovers. On sweet clover (*Melilotus alba*) affected with mosaic the leaves have a mottled appearance and are distorted and curled. The plants are somewhat dwarfed and exhibit an unhealthy appearance. In severe cases an early defoliation is noticeable. The virus appears to be sap-transmissible and passes a Chamberland candle of unspecified porosity (31). The virus can be inoculated to red clover (*Trifolium pratense*), on which the symptoms are very severe, to broad bean (*Vicia faba*) and spotted burr clover (*Medicago arabica*), the incubation period in the plant being ten to fifteen days. No infection could be obtained on white clover (*Trifolium repens*) or on alfalfa (*Medicago sativa*) (10). The insect vector of the clover mosaic virus appears to be the pea aphis *Macrosiphum pisi* Kalt. Van der Meulen (33) considers that *Aphis rhamni* is also a vector of clover mosaic.

Garden Pea (*Pisum sativum*), **Sweet Pea** (*Lathyrus odoratus*). These two plants are commonly affected with a mosaic disease which is almost certainly due to the same virus, since cross inoculations between the two species can be carried out, producing in each case the symptoms specific for the species. On the garden pea, the disease is characterized by a distinct mottling of the leaves with little wrinkling or distortion. Mosaic pea leaves are usually a lighter green than normal, and, in most cases, the mottling is due to the presence of numerous small dark green areas which occur between the larger veins. These dark areas are irregular in outline and usually seem to follow the veinlets. In long-affected plants the dark green portions of the leaves may be replaced by yellowish green areas. The leaves of mosaic-affected peas are generally somewhat smaller than those of normal plants, and occasionally there is some slight curling of the edges of the younger leaves. The incubation period of the virus in the plant is six to fourteen days.

In the sweet pea the symptoms are more severe: at first the general appearance of the disease is similar to that in the garden pea, but later the young leaves develop a pronounced upward curling of the edges giving them a rolled appearance. Such leaves commonly develop small elongate areas light yellow in colour and thinner than normal. These areas may be raised or depressed. The blossoms are streaked and paler than normal. The virus is sap-inoculable and the insect vector is the pea aphis *Macrosiphum pisi* Kalt. The disease is common in Europe and the U.S.A. The writer has seen it occurring generally throughout Cambridgeshire.

The virus of red-clover mosaic has been transmitted to the garden pea and sweet pea by artificial inoculation and to the garden pea by the aphis. Inoculation by the aphis has indicated that red clover is susceptible to the mosaic virus occurring on these two hosts. Cross inoculations from mosaic sweet clover (*Melilotus alba*) and bean (*Phaseolus vulgaris*) to garden and sweet peas were unsuccessful (9).

The mosaic diseases of the sub-order *Papilionaceæ* as a whole have been studied by Merkel (32), *i.e.*, beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), peas (*Pisum sativum*), sweet peas (*Lathyrus odoratus*), clovers (*Trifolium* and *Melilotus*), lucerne (*Anthyllis vulneraria*) and yellow lupins (*Lupinus luteus*). The symptoms described by Merkel comprise sprinkle, *i.e.*, leaves dotted with irregular patches independent of the veins, vein mosaic, mottling and pock mosaic in which dark green protuberances of irregular extent develop on the pale green leaves. In addition to *Macrosiphum pisi*, Merkel considers *Aphis rumicis* (= *fabæ*), the black bean aphis, to be a vector of the virus. The results of Merkel's investigation lead him to the conclusion that the same virus is responsible for all the mosaic diseases of the Papilionaceæ. Some recent cross-inoculation experiments by Zaumeyer may throw some light on this latter point (56). These have shown that the viruses causing mosaic disease in the following plants are capable of being transmitted to beans: white sweet clover, *Melilotus alba*; yellow sweet clover, *M. officinalis*; white clover, *Trifolium repens* and alsike clover, *T. hybridum*. Negative results were obtained from mosaic red clover, *T. pratense*. The virus of the mosaic of white sweet clover produced symptoms on beans slightly different from those of the common bean mosaic. The mosaic virus of garden and sweet peas gave positive results when inoculated into beans either by means of expressed juice or by the pea aphis *Macrosiphum pisi*. This last result is the reverse of that obtained by Doolittle and Jones (9) in their inoculations from mosaic-affected garden and sweet peas to beans (*Phaseolus vulgaris*), but Zaumeyer does not state what variety of beans he used.

While the mosaic diseases of the various clovers and of sweet and garden peas are probably due to one virus, in the writer's opinion the mosaic disease of the bean *Phaseolus vulgaris* L. is due to a different virus, and on this assumption it will be separately discussed.

Phaseolus vulgaris L. (French bean, snap bean). The mosaic disease of this plant has been extensively studied, notably in recent years, by Fajardo (11) and Ray Nelson (34). The symptoms of mosaic upon *P. vulgaris* are variable and are influenced by environmental conditions. In general, the leaves show varying degrees of mottling or chlorosis, blistering and downward cupping of the lamina. Under certain environmental conditions not usual in the field, much-reduced leaves with sinuous or filiform leaflets are produced; in some cases only the terminal leaflet develops. Plants are rarely killed by the virus, but become stunted, dwarfed and bushy. In the season of infection the first signs of the disease show on the young expanding leaves eight to fifteen days after infection. The youngest leaves become crinkled, chlorotic and stiff with a much shortened petiole. The next youngest leaves are at first thickened, more or less chlorotic and laterally arched, later becoming completely chlorotic. The virus of bean mosaic is seed-transmitted (see p. 197) and seedlings arising from infected seed may develop typical mottling, blistering and downward cupping, etc., on the first and succeeding compound leaves, while the simple leaves are chlorotic and mottled or else normal in appearance. There is considerable variation in the time of appearance of symptoms in seedlings arising from infected seed (11). The virus of bean mosaic is sap-inoculable and the insect vectors are said to be the aphides *Macrosiphum gei*, *Myzus persicæ* Sulz. and *Aphis rumicis*. As already mentioned the virus is carried in the seed. Ray Nelson (34) has made a cytological study of mosaic-affected beans and he finds that disintegration of the chloroplasts is frequently to be observed in the palisade and mesophyll cells, and this may result in the complete destruction of the plastids. A coccoid-like organism appears also to be concerned with the disintegration (see p. 259).

The physical properties of the bean mosaic virus are as follows (12): it retains its infective power for twenty to twenty-four hours in extracted sap at room temperatures, and the dilution end-point is about 1/1000. The virus is inactivated by treatment with 25 per cent. alcohol for thirty minutes and with 75 per cent. alcohol for less than five minutes; it is very sensitive to desiccation, being inactivated in seedlings allowed to dry at room temperature for seventy-two hours. The thermal death-point lies between 44° C. and 56° C., but the virus seems able to withstand slightly higher temperatures when in the seed. Freezing does not affect the viability of the virus, and it appears unable to pass any grade of Berkfeld filter.

The mosaic disease of beans probably occurs wherever beans are grown, being distributed with the seed. It was first described by Iwanowski in Russia in 1899 (22). It has been reported from Sweden, Holland and England, and is widespread in the United States of America. It has also been reported from Bermuda and Eastern China.

A disease which may be due to a different virus from that causing mosaic in *Phaseolus vulgaris* has been described (34). This is known as " rugose mosaic," and has only been observed in the American variety " Stringless Refugee." The leaflets of affected plants are usually dark green in colour and are savoyed in appearance, which is the most characteristic field symptom. The pods also may present a ruffled appearance. The disease has not been artificially transmitted.

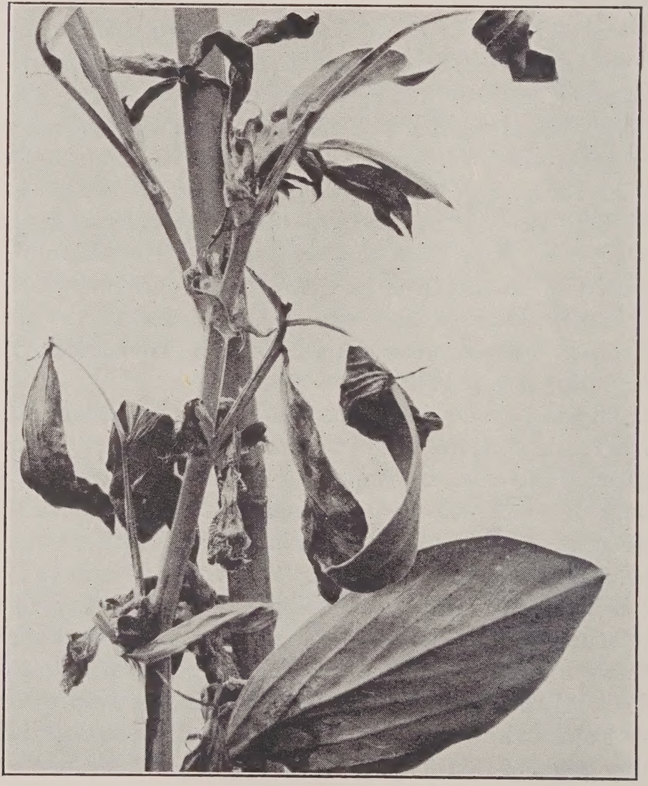

FIG. 56.—Broad bean (*Vicia faba*) affected by the virus of tomato spotted wilt. Note the characteristic zoned lesion on the leaf at the base of the plant.

According to Zaumeyer and Wade (57), the viruses causing mosaic in white sweet clover (*Melilotus alba*), yellow sweet clover (*M. officinalis*), white clover (*Trifolium repens*), alfalfa (*Medicago* *sativum*) and sweet pea (*Lathyrus odoratus*) are all transmissible to beans (*Phaseolus vulgaris*), while the virus producing mosaic in red clover (*Trifolium pratense*) is not.

Beans are also susceptible to attack by viruses more usually associated with other plant hosts. Thus, many varieties of beans, including *Phaseolus vulgaris*, also clovers and alfalfa, are susceptible to a tobacco ringspot virus described by Wingard (55). The present writer has shown that the broad bean (*Vicia faba*) and the cultivated lupin are susceptible to infection with the virus of tomato spotted wilt (46) (see Fig. 56).

A virus disease of the garden pea, *P. sativum* L., characterized by streaking and brown necrosis of pods, stems and leaves, has been recorded from America. The virus is transmitted by *Thrips. tabaci* and is thought to be the same infective agent as that causing " yellow spot " of pineapples (28). The virus of a tobacco mosaic produces local lesions on the leaves of *Phaseolus vulgaris*, but does not give rise to systemic infection (41).

Arachis hypogæa L. (Peanut or groundnut). The peanut is liable to attack by a virus producing a disease known as *rosette* (49). The disease produces a striking modification in the peanut plant ; the whole plant may be little more than a close tuft of small curled leaves, forming a cushion of a few inches in diameter, or its branches may be of some length but bearing terminally, similar tufts of small leaves. Accompanying the rosette form of symptom is a yellowing of the young leaves, which may be separated off by normal green tissue giving a mosaic appearance (see Fig. 57). The first sign of the disease is a faint indefinite mottling of the youngest leaflets. The next leaves to appear are of a pale yellow colour, upon which the veins form a green network—this character is of some diagnostic value. Later-formed leaves bear progressively smaller leaflets, chlorotic, curled and distorted. The virus of peanut rosette is not sap-transmissible, nor is it seed- or soil-borne ; the insect vector is the aphis *Aphis leguminosæ* Theo. Hayes (19), working with this disease in the Gambia, suggests that *Aphis laburni* may be an additional vector. An infrequently occurring mosaic-like form of the disease is thought to be an exceptional reaction on the part of individual plants to the rosette virus and the mosaic disease described by McClintock may therefore be due to the same virus.

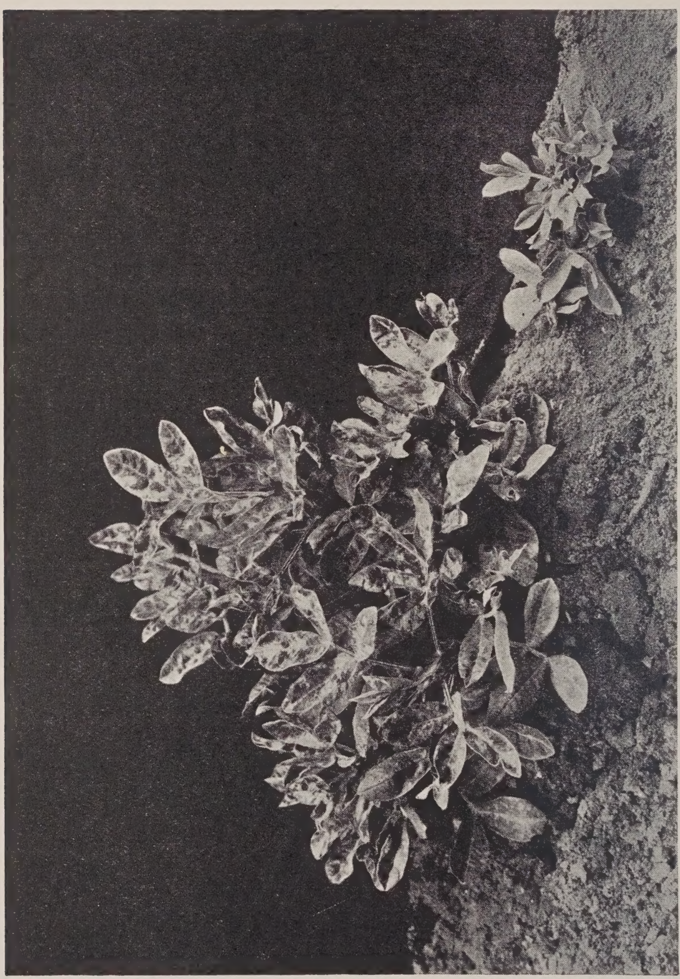

Fig. 57.—Rosette disease of peanuts (*Arachis hypogæa* L.). The plant on the right shows typical rosette, that on the left shows the mosaic type of the disease; plants of the same age and infected at about the same time. (After Storey and Bottomley.)

Rosette of peanuts has been recorded from South and West Africa, Java and India. Hayes (19) describes three types of rosette disease of groundnuts in the Gambia, two of which he thinks may be due to different virus entities. The first type is a chlorotic rosette characterized by light yellow and pale green spots ; the affected leaves are narrower than normal and almost sessile. The internodes also are shortened. The flowers which fail to open are reduced in size, and sessile. The second type, or green rosette, shows no chlorosis and the leaves are darker than usual with a roundish appearance. In the third type the leaves are normal in colour but smaller and thicker, the stems also are thicker and the branches curve in a clockwise direction.

The writer has received from Dr. Pethybridge a lupin which shows symptoms of a mosaic disease, the leaves are mottled with light and dark green patches, and there is a tendency to chlorosis. Flowers are seldom formed. The virus appears to be transmissible by sap-inoculation to tobacco plants, White Burley variety, on which it produces transitory mosaic symptoms. Subsequently the tobacco plants appear to recover.

Rosaceæ (*Prunus*). A serious virus disease of the plum known as " plum pox " has been recently described (2). Primary symptoms take the form of characteristic light green or yellowish green spots, blotches or rings upon the leaves. Diseased trees blossom and set fruit normally. When the fruit has reached normal size, one or more blue spots develop, which often have the form of arches or rings. These spots become sunken and give a pock-marked appearance to the fruit. Under the pock marks the tissue is frequently necrotic, and the necrotic areas may extend down to the stone and become pockets filled with gum. Affected fruits have a low sugar content, are tasteless and shrivel prematurely. Diseased trees degenerate slowly, the smaller branches and annual shoots begin to die and the trees take on a sickly appearance. The virus is communicable by budding and occurs commonly on the native Kustendil plum (*Prunus domestica*) in Bulgaria. Natural methods of transmission are not known. Plum pox is widely spread throughout Bulgaria, except in the plum-growing region in the north. What is apparently the same disease has been recorded in Kentucky, Minnesota, Illinois, Holland, Czechoslovakia, Australia and South Africa.

Cherry. The cultivated cherry is susceptible to a virus disease known as " buckskin," which has caused considerable loss in the cherry-growing district of northern California. The fruits show the most conspicuous symptoms of the disease, being more or less conical in form and failing to mature. Just prior to ripening the diseased fruits cease to develop and the surface of the blossom end takes on a dull " buckskin " appearance. During early autumn the leaves on infected portions of the tree show a peculiar orange to maroon coloration along the base of the midrib and basal lateral veins. The disease is graft-transmissible, but other methods of transfer are not known (42). The disease may be due to the same virus as that causing the preceding " plum pox." The cherry tree is also susceptible to some of the viruses more particularly associated with the peach.

Peach. The peach tree is liable to attack by several viruses, and these will be dealt with *seriatim.*

Mosaic. An apparently new mosaic disease of the peach has recently been recorded in Texas. The majority of fifty-six healthy nursery trees grafted or budded with mosaic material in July 1931 showed similar symptoms early in 1932. These symptoms appeared not only on the new growth from the aerial parts of the trees but also on newly developed root suckers. The disease was communicated by grafting from either the roots or shoots of the suspected trees. The virus is therefore evidently systemic. The outstanding symptoms are striking mosaic patterns in young leaves, an abundance of small crinkly leaves with wavy margins, short internodes, clusters of twigs somewhat resembling witch's broom and irregular " bumpy " fruits. The mosaic character of the leaves may be fugitive and the twigs behave variously in growth characters. The insect vectors, if any, are not known (21).

Peach " Phony " Disease. With the onset of the disease a " phony " tree develops shortened internodes, a number of lateral twigs, and large, flattened dark green leaves, giving the appearance of compact, dense growth with very healthy foliage. In young trees, especially, decided dwarfing results and normal trees quickly exceed " phony " trees in circumference and height. The annual terminal twig growth of a " phony " tree may be only from 1 to 6 inches as compared with the terminal growth of 1 to 3 feet or more in normal trees. In affected trees, because of the condensed appearance of the growth, the disease is usually evident at blossoming time and with the first mature leaves in spring. It appears to be an established fact that the " phony " disease is communicable in nature by some means not yet understood, and that nursery trees in close proximity to " phony " trees may contract the disease.

The virus of " phony " disease apparently resides only in the roots, and this fact explains the failure to communicate the disease when buds or scions from affected trees are employed in grafting. The virus can be transmitted by root grafting.

The disease was first recorded from Marshallville, Georgia, about fifty years ago, and it now occurs in the districts which produce 90 per cent. of the commercial peach crop of Georgia. It has also recently appeared in eastern Alabama. " Phony " disease does not appear to have been recorded from Europe (20).

Peach Yellows. A characteristic symptom of peach yellows is the tendency of the leaf to fold together lengthwise along the midrib, giving a " V "-shaped appearance. The midrib further bends downward and inward, resulting in a claw-like or hooked appearance. The leaves are turgid and brittle. The foliage is paler than normal, and the yellow colour may be outstanding in well-advanced cases of the disease. Slender wiry shoots often sprout from the main branches and assume an upright growth even when they originate from the side or underside of a branch. These so-called " wire shoots " have small narrow willow-like leaves of a pale yellowish green colour. The leaves also are hard and leathery owing to the accumulation of starch which is not transported to other parts of the plant. The fruit ripens prematurely, from several days to six weeks in advance of normal. Red blotches appear on the skin and are often associated with red streaks extending into the flesh radially toward the pit. The virus is transmissible by budding or grafting from stem or root; it is not sap-inoculable (7). The mode of transfer of peach yellows in the field has been a matter of conjecture for many years, and it is only recently that an insect vector of the virus has been discovered. Kunkel (27) has shown that the leaf hopper *Macropsis* *trimaculata* Fitch, is a specific vector of the virus of peach yellows. This disease, which does not seem to have been recorded in Europe, is widely spread in the U.S.A. It was first recorded in Michigan in 1863, since when it appears to have become established in all the peach-growing districts of that State.

Little-Peach. In "little-peach" the characteristic symptoms are reduction in size and delayed ripening of the fruit. The tree is often somewhat stunted, with pale yellow leaves and a more compact bushy appearance to the tree. The centres, instead of being somewhat open, are usually filled with shoots covered with clusters of leaves and short lateral branches. The terminal growth of the main limbs in advanced cases is also of this general type. Fruits ripen from a few days to three weeks late. They are reduced in size sometimes, but at other times are full size and ripen only a few days late. The flavour is usually poor. The variety Gold Drop seems to show the greatest delay in ripening and most marked reduction in size. On the other hand, diseased fruits of variety Rochester have shown less reduction in size and ripening is delayed not more than four or five days (3). In the initial stage of the disease the foliage is of a deeper green than normal. The large dark green leaves near the terminal shoots show a drooping (not wilting) appearance and lie closely appressed and almost parallel with the shoots from which they originate. The downward curl of leaves on trees affected by little-peach consists of a bending of the midrib downward and inward, although the halves of the leaves do not usually fold together as in peach yellows (7). The insect vectors are not known. The disease, little-peach, seems to be confined to the U.S.A., and is very prevalent in Michigan.

Peach Rosette. The virus of peach rosette is communicable by grafting to apricots ; cultivated plums ; wild plum ; cherry ; and almonds. On some hosts the virus produces a mosaic mottling on the leaves. The Marianna plum appears to be immune to the virus of peach rosette. There is no evidence of soil transmission, the virus is not sap-transmissible and the insect vectors are not known. Peach rosette appears to be found only in the U.S.A. (30), but it does not occur in Michigan.

Red Suture. An apparently new virus disease of peach trees has been recently described; this is known as "red suture," and is prevalent throughout the peach-growing regions of Michigan (7). The symptoms are most apparent at ripening time and a characteristic symptom is seen in the fruit. These ripen several days prematurely, one side of the peach, usually the suture or crease side, softening first. The peach itself may have a bumpy or rough contour, with heavier ridges usually running lengthwise with the suture, or the ridge of the suture may be abnormally raised. Affected fruits usually show an abnormal deep red to purple blush over the exposed side, and the colour is deepest on the apices or peaks of the ridges. When red suture is apparent in the fruit, the entire tree usually presents a yellowish green or bronzed appearance. The tip is of a lighter colour than the rest of the leaf and there is a certain amount of curling or twisting of the leaves and petioles. Terminal growth is shorter than normal. Leaf clusters often sprout from most of the buds on a branch, and an unusual number of short shoots arise along the main branches towards the base. Such shoots, together with the abnormal number of leaf clusters sprouting on the main limbs near the centre of the tree, give the characteristic symptom described as "feathering" or "fuzziness," and this is not unlike a similar condition sometimes occurring in "little-peach." The methods of transfer of this virus from diseased to healthy trees in the field are not known.

Several of the foregoing virus diseases of the peach are transmissible by grafting or budding to the wild and cultivated plum or to the cherry. It should be realized that peach yellows is due to a different virus from that causing aster yellows (see p. 362), although the latter is transmissible to one species in the Rosaceæ.

Raspberries (*Rubus* spp.). Some uncertainty exists at the present time with regard to the actual number of viruses which are associated with the raspberry and allied species, and this uncertainty applies particularly to the mosaic group of viruses. There are three main types of virus disease affecting raspberry, *i.e.*, curl, mosaic and streak, to which may be added a virus disease described on blackberries and loganberries and known as "dwarf."

Curl. This disease is common in the U.S.A. on red, hybrid and black raspberries, and also on blackberries. On susceptible varieties the characteristic symptoms consist of a curling of the leaves accompanied by the production of a deep green foliage and by a stunting of the entire plant. The curling of the leaves seems to be due to retardation in growth-rate of the veins, while the intervening tissue continues to grow at a more or less normal rate, resulting in the apparent sinking of the veins, a downward curling of the leaf margins, and the production of folds or crinkles in the other parts. The leaves and canes of curl plants appear dry and do not wilt so readily as do those of normal plants. Curl symptoms resulting from the current season's infection often appear on the tips of the new canes by July 1st, and may become characteristic and marked even in the early stages.

Two curl viruses or strains of curl viruses have been described and are designated respectively alpha- and beta-curl viruses. The alpha-curl virus which affects the Cuthbert variety of red raspberry in Michigan, apparently will not produce infection in the curl-susceptible Cumberland variety of black raspberries. The beta-curl virus affects both varieties. Transmission of the two curl viruses has been obtained only with the insect *Aphis rubiphila.* No curl infection was obtained with the aphides *Amphorophora rubicola, A. rubi* or *A. sensoriata* (4).

Streak. Diseased plants present an appearance which suggests mild leaf curl symptoms. Plants are stunted after the first year and become smaller each succeeding season. Leaves on such plants are nearly always distinctly curled and usually placed close together on the canes. The foliage is a shade darker green than normal. Leaves, especially those at the tip of the canes, have a peculiar twist of the midrib, with a decided tendency to bend at an angle and recurve in such a way as to bring uppermost the dorsal side of the tips of the leaflets. Mottling is an uncertain character of streak and may or may not be present. Streaking or striping of the stems of new canes is a fairly constant symptom under certain conditions. Bluish violet, discoloured areas varying from small spots to stripes of differing length and width appear on the main canes and laterals. Streak disease of raspberries has not been transmitted experimentally and the common species of aphides which transmit curl and the mosaic viruses apparently are not vectors of this virus (4).

Mosaic. Mosaic symptoms on raspberry plants vary so much according to temperature relations, varietal reactions and other factors that a determination of the number of viruses involved is extremely difficult. The classification and number of mosaic viruses given here are according to the work of Bennett (6), who states that " it seems questionable whether the present state of our knowledge justifies the use of virus or viruses as a basis for classification in this group of symptoms . . . which will be called red-raspberry mosaic. If there is more than one virus involved it must be left to future research to perfect a method for differentiation and identification." The following are the mosaic types and their symptoms as given by Bennett.

Yellow Mosaic. Cumberland Variety. Plants more or less stunted and decidedly yellow in appearance. Leaves yellow, veins often lighter in colour, symptoms marked during most or all the season, mottling not conspicuous or of a very coarse pattern; cane tips and petioles not necrotic.

King Variety. Plants usually stunted and yellow: leaves with yellowish cast, leaflets rolled downward and inward, mottling slight or of a very coarse pattern. The symptoms are not masked by summer temperatures.

Red-raspberry Mosaic (Mild). Cumberland. Mottling mild but distinct on first leaves of fruiting canes in the early spring; no symptoms are visible on foliage produced later in the season, plants not stunted.

King. Mottling very mild or not evident, restricted to first leaves of fruiting canes, plants not dwarfed.

Red-raspberry Mosaic (Medium severe). Cumberland. Mottling distinct on first leaves of fruiting canes, leaves sometimes distorted. The leaf petioles and cane tips become necrotic in recently infected plants. Necrosis is slight in plants in the second year of disease. Rings of mottled leaves are often found on first-year canes marking periods of low temperatures. The plants are somewhat stunted.

King. Mottling consisting mostly of faded blotches, mild but clearly evident, which develop on the first leaves of the fruiting canes. The plants are not dwarfed.

Red-raspberry Mosaic (Severe). Cumberland. Mottling not excessive, the leaves are often deeper green than normal. The leaf petioles and cane tips are necrotic and brittle. The canes are short and rosetted, sometimes producing normal shoots during periods of high temperatures. The plants are very much dwarfed and are killed in two or three years.

King. Mottling is conspicuous on leaves formed at low temperatures and masked on those formed at higher temperatures. The plants are little, if at all, stunted.

Mixtures of these viruses occur in the raspberry in the field; striping or streaking of the stems of black varieties is produced by a combination of yellow and red-raspberry mosaic viruses.

Some unusual symptoms of virus diseases in raspberries have been described by Zundel (61). Canes were found with a " spindle-sprout " type of growth forming a " witch's broom." This is believed to be an unusual form of raspberry " streak." Zundel also describes a disease known as " fern leaf," which he suggests may be due to infection with the virus of cucurbit mosaic (cf. Mogendorff, tomato fern-leaf, p. 142).

Raspberry mosaic viruses are not apparently sap-inoculable and the chief agent of dissemination is considered to be the aphis *Amphorophora rubi*, while *A. rubicola* and *A. sensoriata* can also act as vectors. *Aphis rubiphila*, the vector of raspberry curl, if capable of acting as a vector of either type of mosaic, probably transmits only in very rare cases.

The foregoing diseases are prevalent in America, but there is little information of their occurrence in Europe; only " raspberry mosaic " has been recorded in England.

Loganberry, Blackberry. A virus disease which has some symptoms in common with streak and yellow mosaic has been described on the loganberry and blackberry and its exact relationship, if any, with these diseases is not known (59). This disease, which is known as "dwarf," is characterized in its severe stages by short, stubby canes, the internodes of which are very short. The whole cane has a leafy appearance. The leaflets are smaller, more rounded and lighter in colour than normal, showing a finely netted uniform mottling. The fruit develops to a fair size, but the druplets easily fall apart, becoming a crumbly mass in the boxes. The virus is not sap-inoculable and the insect vector appears to be the aphis *Capitophorus tetrarhodus* and possibly the aphis *Macrosiphum dirhodum.*

The disease was first recognized in the Pacific Coast States in 1918, and is widespread from British Columbia to Central California. There appear to be no records of the virus in Europe.

Strawberries (*Yellows, Xanthosis*). The most conspicuous symptoms are a characteristic curling and cupping of the leaves accompanied by a yellowing of the margins and between the larger veins. The leaves, both petioles and laminæ, are dwarfed and there is a general stunting of the growth of the entire plant. Other symptoms include a premature reddening or autumnal colour of the older outer leaves and the appearance of small red dots on the lobes of the margins in very young leaves. The virus is transmitted by the runners. The root system does not appear to be affected in the earlier stages of the disease. The virus is not sap-inoculable and is not carried in the seed. The insect vector is the aphis *Myzus fragæfolii.* Air temperatures of 80° F., and over, induce partial or complete masking of the symptoms. The palisade cells of the chlorotic areas of the leaf are shorter and more compact than normal. In the later stages of the disease there arises degeneration of the pericycle region of the roots.

Two types of black-staining intracellular bodies have been described—these show no structure and are considered to be degeneration products. The disease has been recorded from the U.S.A., but there is little information on the virus diseases of strawberries in Europe (38, 39).

Dwarf. Another apparently distinct virus disease of the strawberry has been described (40). This is called "strawberry dwarf," and is characterized by severe stunting and dwarfing of the plant. The leaves become greatly reduced and are strikingly deformed. The leaflets are elongated, unsymmetrical and usually crinkled, the margins are upwardly cupped in the young leaves and downward in other leaves. Chlorosis of the leaves, which is characteristic of strawberry yellows, is absent in strawberry dwarf. The insect vector appears to be *Aphis forbesi* Weed. Intracellular inclusions, similar to those described from strawberry yellows, occur in the pericycle cells of young roots of dwarfed plants.

Yellow Edge. A virus disease of strawberries in England has recently been described (18). In this disease, which has been termed " yellow edge," the leaflets exhibit a chlorosis or yellowing confined to the marginal regions and invariably accompanied by the following symptoms : (*a*) a general dwarfing ; (*b*) an irregular curling, usually in an upward direction, of the marginal region ; (*c*) a downward curling of the midrib and (*d*) a twisting of the whole lamina.

A complete leaf has a distorted and asymmetrical appearance due to the constituent leaflets showing the above symptoms in varying degrees. The petiole is abnormally short, stout and lacking in red pigmentation. Premature autumnal discoloration is induced in infected plants.

The disease is very similar to the Xanthosis of strawberries described above.

Witch's Broom. Symptoms of this disease differ somewhat according to the variety of strawberry and may be divided into two main types. In the first type, exemplified on variety Marshal, diseased plants have long, usually erect, stiff, spindly petioles, upon which are borne leaflets which are much smaller than those of healthy plants. The leaves are usually of a light olive-green shade and the mid-veins of the leaflets arch downward. This tendency to curve downwards is in direct contrast to the upward cupping of the leaflets in strawberry yellows. The great number of leaves with long petioles give the bushy appearance which suggests the name witch's broom for the disease. In the second type of symptom which appears on strawberry varieties of a more bushy habit of growth, the brooming character of the disease is more pronounced. In this type of plant the internodes of the runners are extremely short and the leaves are not arched downwards at the edges to the same extent as in the first type of symptom. The virus is not apparently sap-inoculable and the insect vector is the aphis *Myzus fragæfolii*. The disease has only been recorded from Oregon, U.S.A. (58).

Roses. There seem to be two types of virus disease affecting the rose: one of these is known as " rose wilt " and " dieback," while the other is an infectious chlorosis or mosaic. In rose wilt the first symptoms consist of a peculiar recurved appearance of the leaves of the young shoots, the leaflets of which sometimes also seem to be crowded together on the petiole, and are very brittle. Defoliation begins at the tips of the stems of affected plants and works downwards, the leaves sometimes turning pale green or brownish before dropping. About a day later the tips of the young stems begin to discolour and die back for a distance of 1 or 2 in. The rest of the young stem then develops a characteristic translucent, yellowish green appearance, the base turning brownish black within a few hours. Young leaf buds in the basal browned area remain green for some time. Gradually the whole stem becomes discoloured and dies back, the developing leaf buds turning brown at the tips and rotting away. In many cases the plant sends up one or more water-shoots after the stems have died back, but they soon become diseased.

The virus has been shown to be filterable through a Seitz filter and is thus transmissible by needle inoculation. The insect vector does not appear to be known. The disease has been described only in Australia (17).

Mosaic. In mosaic disease of hybrid tea roses, the plants are dwarfed, the degree of dwarfing depending upon the variety and environmental conditions. The dwarfing is expressed in all parts of the plant, including the roots. Buds are often imperfect, on short stems and bleached. The normal colour of the flowers in certain varieties is affected. The leaves are variously distorted, with the midrib frequently bent and twisted. The leaflets show distinct chlorotic areas, especially along the midrib, which cause the leaflets to pucker and ruffle, while clearing of the veins is frequently pronounced. Usually all leaflets on a leaf show symptoms, but sometimes one leaflet of normal size and without symptoms may occur while the rest of the leaflets show marked symptoms. When the chlorosis is confined to one side of the leaf, distortion occurs due to unequal growth of the two halves (54). Symptoms vary considerably according to the variety, ranging from minute speckling and occasional distortion to those symptoms above described. On *Rosa manetti* the symptoms occur as numerous, minute chlorotic areas, distributed over the entire leaflet or concentrated toward the tip or periphery of the leaflet, with some of the chlorotic specks apparently interveinal (53).

The virus of rose mosaic, unlike that of rose wilt, appears to be transmissible only by grafting. The insect vectors are not known. The disease is prevalent in the U.S.A. and is probably also present in Europe, and the present writer has had cases of what was apparently this disease reported to him in England.

Holodiscus discolor Max., (Ocean Spray). An apparent virus disease of this ornamental shrub has been described (60). The disease is known as witch's broom, and when it first makes its appearance on the plant the new lateral branches from an old stem are very slender and wirelike, with rather short internodes and small leaves. New canes, which arise from or near the crown after the plant has become affected, are short and have a stiff appearance. In these canes the internodes are short ; the main stems have little tendency to branch, so that there are usually no blossom clusters. The leaves of affected plants are small and crowded, giving the canes a very leafy appearance. Where they are not shaded they turn a bronzy red early in the summer. This colour stands out in contrast to the bright green of neighbouring healthy plants. The disease is transmissible by grafting but not by sap-inoculation ; the insect vector appears to be *Aphis spiraeœ*. Witch's broom of *H. discolor* has been recorded only from the U.S.A. It has been found in the foothills of the western slope of the Cascade Mountains, from Linn to Wasco counties in Oregon and in Thurston County, Washington.

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CHAPTER XIV

THE VIRUS DISEASES OF PLANTS (*Continued*)

Cucurbitaceæ

Cucumber. Apart from the true tobacco mosaic viruses, some at least of which readily infect the cucumber, there are several mosaic viruses which may be considered as primarily associated with this plant. There are at least three if not more such viruses recorded in the U.S.A., while the cucumber mosaic virus occurring in England is probably still another entity. According to James Johnson's system of nomenclature and differentiation (44) cucumber virus 1, as described by Doolittle (17), produces on the cucumber plant chlorosis, mottling, stunting, malformation and necrosis.

First symptoms appear in the young leaves in which small greenish yellow areas develop. Less commonly the normal green of the leaf changes to a peculiar yellow at the tip. Accompanying these symptoms there is a gradual downward curling of the edges of the leaf and the surface presents a finely wrinkled appearance, the tissue between the small veins becoming slightly raised so as to form minute convex surfaces. Following these early symptoms, the gross mosaic symptoms such as a pronounced green and yellow mottle, accompanied by wrinkling, distortion and savoying, develop. All growth subsequent to infection is much dwarfed, the stem internodes are shortened, the leaves only attain about one-half normal size, and the petioles are reduced in length. Marked symptoms develop on the fruit which first becomes mottled with yellowish green. As this progresses, the body of the fruit ordinarily becomes a light yellowish green, intermingled with spots of a much darker green. These dark portions are usually raised above the surface in such a way that they form wartlike projections and often produce more or less distortion of the fruit (17).

With regard to differential hosts, it produces on *Nicotiana glutinosa* mottling, stunting and malformation; on pokeweed, mottling and stunting, while on tobacco it has a chlorotic effect but as a rule no malformation. Affected tobacco plants have a general chlorotic appearance with occasional dark green puffed areas. The upper leaves are narrower than in normal plants and may be pinched at the tips. Necrotic ring-like patterns sometimes develop on inoculated leaves. The incubation period of the virus in tobacco plants is about a week or slightly longer (43). The symptoms of cucumber virus 1 on tobacco can be differentiated from those of most tobacco mosaic viruses by the larger chlorotic areas produced by the former, usually near the tips of young leaves. Cell inclusions are not associated with this virus. The virus is apparently transmissible only through the seed of the wild cucumber, *Micrampelis lobata* (20); it is sap-inoculable.

The host range includes many members of the Cucurbitaceæ and Solanaceæ; it has also been transmitted to spinach, on which it produces the disease known as "spinach blight" (39), pokeweed, *Martynia louisiana*, and pigweed, (*Amaranthus retroflexus*). On tomato this virus frequently produces the "fern leaf" type of symptom.

The insect vectors appear to be the aphides *Myzus persicæ* Sulz., *Macrosiphum gei* Theob., and *Aphis gossypii* Glov., and the leaf beetles *Diabrotica vittata* and *D. 12-punctata*. As regards physical properties, the longevity *in vitro* is 3 days or less, its thermal death point is 60–70° C. after ten minutes' exposure and it is inactivated by one hour's exposure to 50% alcohol or 1 : 200 nitric acid (44). It is killed by desiccation.

Cucumber mosaic is widespread in the U.S.A., where it has ruined the cucumber-growing industry in certain parts. E. M. Johnson (43) describes two other apparently distinct cucumber viruses under the designations "cucumber mosaic types 2 and 3," but the symptoms on cucumber are not given. In type number 2 on tobacco the most conspicuous symptom is a mottling formed by the development of pale green or yellow tissue along the veins. In the third type the symptoms are similar to those produced by cucumber virus 1 but are more prominent ; they can be differentiated by the drooping yellowed lower leaves and malformed bud leaves characteristic of the former virus.

An apparently new mosaic disease of cucumbers has recently been described (70). The symptoms of this disease, or " Bettendorf " mosaic as it is called, on cucumbers are rigidity of the terminal leaves with yellow blotches—at first distinct, later merging together. No visible symptoms appear on the fruit and this contrasts with the behaviour of cucumber virus 1 where the fruits are markedly affected. The virus of Bettendorf mosaic can be distinguished from cucumber virus 1 by symptoms, period of incubation and by means of differential hosts. Thus, while the former virus has an incubation period of six to fifteen days and will infect watermelon and the resistant cucumber variety Chinese Long, the latter is stated to have an incubation period of three to six days and to be unable to infect watermelons and the Chinese Long cucumber.

Vegetable Marrow. A mosaic of this plant has been described by Ogilvie (67) and the present writer has also worked with this virus. The symptoms take the form of a very bold mottling of the leaves, with pale yellow markings which tend to run in wavy lines or circles. The leaves are somewhat puckered. Affected shoots have decidedly shortened internodes and a tendency to branch. The fruits of diseased plants are mottled and covered with somewhat circular wart-like areas ; much of the young fruit dies off without developing. The virus is transmissible by sap-inoculation to the Solanaceæ and the writer has successfully infected various members of this group. On tobacco the symptoms consist of a faint light green mottle not at all like the disease produced by cucumber virus 1 on that plant. A similar effect is produced upon *Datura*. On *Nicotiana glutinosa*, however, the symptoms are strongly marked. The leaves show a uniform mottle of light and dark green. Growth is inhibited and the plant becomes dwarfed, bearing numbers of short stems arising out of the top of, and almost at right angles to, the main stem. These short stems bear small distorted leaves. The disease tends towards a rosette effect. The vector of the virus is probably *Macrosiphum gei*.

Saxifrageæ

Ribes. There is little information available on the viruses affecting members of this family. The condition known as " reversion " in black currants seems to be connected in some way with the " big-bud " mite, *Eriophyes ribis*, and it is possible that reversion is a disease of the virus group disseminated by this mite (2). Rosenthal (75) gives a note on the occurrence of reversion in black currants under the title of " Virus diseases of the black currant," and considers it is responsible for much sterility in currants in Germany.

Umbelliferæ

Apium (Celery). There are records of three viruses affecting the celery plant. In *celery mosaic* the symptoms are conspicuous and the entire plant is usually affected, the foliage sometimes drooping, wilting or spreading. In most cases diseased foliage is nearly erect or straight, becoming filiform and producing a bushy top. As a general rule the leaves are shrivelled and show numerous blister-like pustules, which apparently do not burst but affect the interior cell structures of the leaf to the extent of causing malformation. The diseased parts are very brittle, especially the young central branches. There is no increase or decrease in the coloration of affected plants. The insect vector is the aphis *Myzus persicæ* Sulz. (69).

The celery plant is also susceptible to one strain of the virus causing aster yellows (see p. 362). The first symptom of this disease, called *celery yellows*, is a vertical position of the petioles, which are somewhat longer than those of the healthy plant. The petioles of the innermost or youngest leaves are shortened and chlorotic and begin to twist and intertwine. A general yellowing of the plant then develops with a premature blanching of the outer leaves. The vertical or upright petioles gradually assume a flat position. The petioles are brittle, break easily and often crack. In the later stages of the disease the heart of the plant decays, forming a soft yellowish brown rot which extends down into the base of the plant (80). The celery is apparently not susceptible to the New York strain of aster yellows but only to that from California (see p. 287).

Doolittle describes a mosaic disease of celery which is thought to be due to the same entity as cucumber virus 1 (18). In this case the insect vector is *Aphis gossypii* Glov. The writer has observed a mosaic disease of celery in England which somewhat resembled the disease described by Poole (69).

Dipsaceæ

Scabiosa. A species of wild scabious has been observed in England affected with a mosaic disease. There is some evidence that this virus is transmissible by needle inoculation, but there is at present no further information concerning it.

Compositæ

Callistephus chinensis Nees (China Aster). A serious virus disease of this plant known as *aster yellows* is prevalent throughout the U.S.A. Aster leaves affected with this virus never show mottling. The first symptom is a slight yellowing or " clearing " of the veins in a single young leaf. After the plant has been infected for some time the leaves become chlorotic throughout. While chlorosis does not appear in leaves that are mature when the plant is attacked, leaves that are from one-half to two-thirds full grown and are of a normal green colour do become diseased, as is shown by clearing of veins and general chlorosis. This would seem to indicate that the virus is capable of destroying chlorophyll. Sheffield, however, in regard to tomato aucuba mosaic, states that this virus does not destroy the chlorophyll in mature leaves but only inhibits the development of the chloroplasts (see p. 38). When an attack of aster yellows is severe, the young leaves are almost white. The virus has the effect of depressing chlorophyll production in portions of the plant that are normally green but causes the production of some green-coloured substance—probably not chlorophyll—in floral parts of the plant where chlorophyll is not normally present. One of the most striking symptoms of aster yellows results from the abnormal production of secondary shoots. Such shoots frequently arise in the axils of leaves that show a normal green colour and were mature before the plant became infected. The degree of stunting varies with the age of the plant at the time it becomes infected and with the size of the sector infected. The effect of the disease on the main stem is greatly to shorten the internodes while having an opposite effect on the secondary shoots. Diseased leaves have a tendency to stand upright instead of lying flat as in normal plants. Necrosis is a symptom of advanced stages of yellows and is most severe in the stem tissues a short distance below the apical bud of the main stem.

In summarizing, the chief symptoms of aster yellows may be said to consist of diffuse and well-marked chlorosis, clearing of veins, occasional one-sided or sectorial infection, upright habit of growth, malformation and increased growth in certain organs, dwarfing of the plant as a whole, and the abnormal production of secondary shoots. The virus is not sap-transmissible and the specific insect vector is the leaf hopper *Cicadula sexnotata* Fall. The host range of the aster yellows virus is remarkably wide and it has been experimentally transmitted to more than 120 host species belonging to 30 different families of plants. It has not been transmitted to potato, tobacco or peach. The disease is widespread in the U.S.A., but has not been recorded from Europe (48, 50, 80).

Lactuca (Lettuce). The lettuce is susceptible to a mosaic disease, the first symptom of which is a yellowish discoloration along the smaller veins of the younger expanding leaves. This symptom persists for a few days only, giving place to a general yellowish, discoloured appearance of the whole plant. Close examination usually reveals irregular blotches of an approximately normal green colour located as a rule along the larger leaf veins. The blotching varies from a few barely perceptible green areas on a yellowish leaf to numerous pronounced green spots giving a marked mottled appearance. The leaves of diseased plants are generally more wrinkled than those of normal plants. In general, affected plants make a stunted growth and in severe cases are decidedly undersized. Occasionally the leaves form only a rosette with no indication of a folding together of the tips to form a head. The aphis *Myzus persicæ* is the vector of this virus (42).

Two types of virus disease in lettuce occur in Germany—one of these, known as " leaf vein," has not been proved to be a virus and will not be dealt with here. The other type is known as "*dot mosaic*," and is characterized by pale or white, sharply defined, spots scattered irregularly over the youngest leaves. In more advanced stages these spots often assume a necrotic greyish brown appearance. Sometimes the leaves are very much crumpled and stunted. The seed-bearers show a pale green to yellowish discoloration of the leaf margin and severe necrosis of the lamina, which may be entirely destroyed. Histological examination of diseased lettuce tissue reveals the presence in the phloem cells of corkscrew-shaped or wavy bodies which seem to involve disorganization of the nuclei. The virus of " dot mosaic " of lettuce is seed-borne to the extent of about 8–10 per cent. The insect vector appears to be the aphis *Myzus hieracii* Kalt. (= *Macrosiphum hieracii* Schout.) (8).

The lettuce is also susceptible to the virus of aster yellows, on which it produces a disease known variously as " white heart," " rabbit-ear " or " Rio Grande disease." The outer leaves of lettuces so affected are yellow in colour, while the heart is dwarfed and blanched and the young leaves are much reduced. In plants infected before heading, the heart leaves curl outward instead of inward and form no heads (80).

Taraxacum (Dandelion). The only record of an apparent virus disease of the dandelion is a short note by Morse (64), who describes a disease which he calls " yellows." The present writer has worked independently with what is apparently the same disease which is widespread in dandelions in the vicinity of Cambridge. Symptoms take the form of bright yellow spots on the leaves, together with the production of abnormal numbers of stems. There is some evidence that the disease is transmissible by needle inoculation, but the insect vectors are not known.

Dahlia. The dahlia is subject to two or more viruses of the mosaic type. The writer has shown (83) that this plant is susceptible to the virus of tomato spotted wilt, which gives rise to concentric rings and patterns on the leaves (see Fig. 58). The vectors of this virus are two species of thrips (see p. 377).

A mosaic of dahlias has been described in Germany. In this disease the young leaves show an irregular pale discoloration, especially near the main and lateral veins, but later the normal tint develops. Affected leaves, however, are smaller and narrower than normal ones and are frequently deformed; certain colour changes may also take place in the flowers of some varieties. According to Brandenburg (8) the virus of this dahlia mosaic is not sap-transmissible nor is it carried by the aphis *Myzus persicæ*.

Intracellular inclusions are associated with mosaic of the dahlia and have been described by Goldstein (30) and Brandenburg (8). A dahlia mosaic is prevalent in New York, New Jersey, Connecticut and probably throughout the U.S.A., but it is not certain whether it is due to the same virus as that causing the disease described by Brandenburg. The most characteristic

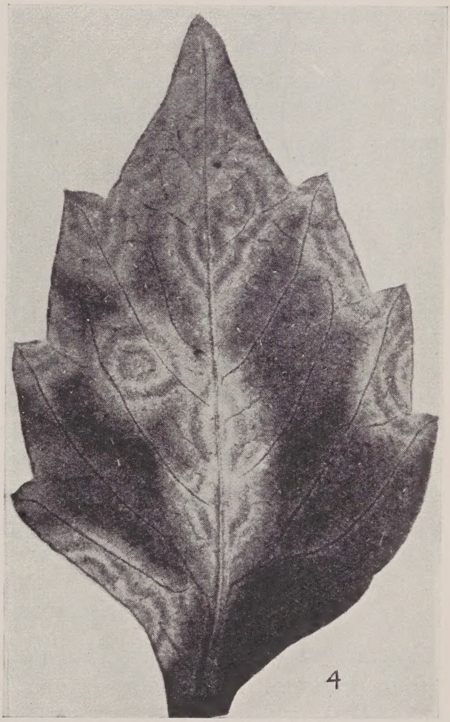

Fig. 58.—Leaf of a dahlia plant affected with the virus of tomato spotted wilt. Here the symptoms are of the ringspot form.

symptom is a banding of the veins. Some dahlia varieties show no appreciable dwarfing or distortion when affected, while others show marked dwarfing, various forms of leaf distortion or vein necrosis. Brierley (10) states that the American dahlia virus is transmissible by *M. persicæ*, in contrast to the statement of Brandenburg that *M. persicæ* is not a vector of the dahlia mosaic. Brierley also finds the virus not sap-transmissible nor carried in the seed. A ringspot of dahlia has also been described in America which is characterized by zigzag or ring markings in shades of green: this is not certainly distinct from mosaic. The viruses affecting the dahlia are carried in the tubers and so perpetuated from year to year.

The so-called " stunt " disease of dahlias is thought to be due to a virus affection. Disease plants are stunted from the beginning and often form an excessive number of shoots. The leaves are curled and numerous small cracks appear in the stem and also in the skin of the tubers, the development of which is much retarded. Affected plants seldom flower. Intracellular inclusions have been demonstrated in the cells of dahlia plants affected with mosaic and " dwarf," which is presumably the same as the " stunt " disease above referred to. The association of intracellular inclusions with this latter disease is strong evidence of its virus nature (30).

Ericaceæ

Vaccinium (Cranberry). This plant is affected with a virus which produces a disease known as " false blossom." The cranberry is a perennial evergreen plant with a vine-like habit of growth. The side runners attain a length of 3 to 5 feet, rooting frequently and making a mat with neighbouring plants. The centre shoots or uprights, 12–18 inches high, continue growth so that the flowers appear lateral. The false blossom disease is most readily recognized when the plant is in bloom. The name " false blossom " is appropriate because the flowers show the symptoms most clearly and are usually rendered sterile. The flower as a whole assumes an upright position instead of the normal curve of the pedicel. The calyx lobes of diseased flowers become enlarged, the petals are short and streaked with red and green, and the stamens and pistils are more or less abnormal. When the disease is severe the entire flower may be replaced by successive whorls of leaves or by a short branch. The leaves also show symptoms of the disease. Axillary buds which are usually latent produce numerous negatively geotropic branches with many crowded leaves which are closely appressed to the stem. The resulting structure is referred to as a witch's broom. In autumn the leaves take on a reddish hue, which enables one to locate a diseased area at a distance. One of the most striking symptoms of false blossom is the production of shoots by axillary buds which in a healthy plant would remain dormant. Cross sections of healthy and diseased terminal buds show that in the diseased bud the flower rudiments are in a relatively advanced stage and only one layer of scale leaves protects them instead of at least four in the healthy buds. The result of this premature development of the terminal fruit buds in diseased cranberries is that the buds are frequently killed by spring frosts.

The virus is not transmissible by mechanical means and the insect vector is the blunt-nosed leaf hopper *Euscelis striatulus* Fall. (according to some authorities *Euscelis vaccinii* Van Duzee). Cranberry false blossom occurs in the U.S.A. wherever cranberries are grown east of the Mississippi River. The disease has not been recorded in Europe (16).

Convolvulaceæ

Ipomœa batatas Lam. (Sweet potatoes). Rosen (74) describes a mosaic disease of sweet potatoes, which he considers to have an incubation period in the plant of not less than two consecutive growing seasons. Typical symptoms are dark green coloration, deep indentation and distortion of the leaves, with a superficial development of white pencilled lines running in all directions. The insect vectors are not known.

Bindweed (*Convolvulus arvensis* L.) is susceptible to the virus of tomato spotted wilt which gives rise to concentric circles on the leaves.

Solanaceæ

The virus diseases of the potato plant have been fully dealt with in Chapter XII., and so are not considered here. In this section only the more important virus affections of the tobacco and tomato plants are reviewed. The confusion regarding the differentiation of the viruses affecting these two plants, while not so acute as in the case of potato viruses, is still considerable, and it will not be possible in the space available to deal fully with all the tobacco virus diseases which have been described. In the majority of cases the various viruses are equally infectious to both tobacco and the tomato, and they will therefore be dealt with under the host with which they are usually associated or on which they were originally described.

Nicotiana tabacum (Tobacco). *Mosaic Viruses.* A virus of tobacco mosaic was the first virus to be discovered and was described by Iwanowski in 1892 (41). There is little doubt that there exist several viruses of the tobacco mosaic type which resemble each other in their main characteristics. Many of the viruses of this group are characterized by extreme ease of transmission by sap-inoculation, high resistance to ageing *in vitro* and a high degree of resistance to temperature, alcohol and certain chemicals. They are easily filterable through candles as a rule. Tobacco mosaic viruses usually cause a pronounced mottle on that plant; this mottling may be quite brilliant (see Fig. 59), consisting of bright green, yellow or white patches, often accompanied by considerable distortion and blistering of the lamina. The first symptom is usually a yellowing of the veins of the youngest leaf.

Little seems to be known of the insect vectors of tobacco mosaic viruses in the field, and it is a remarkable fact that so infectious a group of viruses should apparently have little connection with insect vectors. J. Johnson (44) has classified nine mosaic viruses affecting the tobacco plant, six of which may be regarded, for the purpose of description, as primarily tobacco viruses. The following facts are from J. Johnson's classification of tobacco mosaic viruses.

Tobacco Virus I. (Type: *Allard, U.S. Dept. Agric. Bull.,* 20, 1914.) The symptoms on differential hosts consist of marked mottling, malformation and stunting on tobacco; stem and leaf necrosis and stunting with no mottling on *Nicotiana glutinosa*; mottling and stunting with no stem necrosis on tomato; no symptoms on pokeweed (*Phytolacca decandra*). For a very comprehensive description of the symptoms of this virus upon a series of differential hosts the reader should consult a recent paper by Holmes (40).

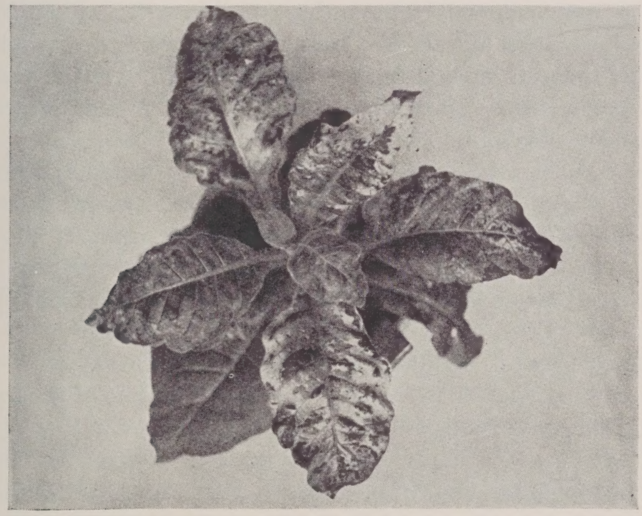

FIG. 59.—Tobacco mosaic. This virus causes the development of large yellow or white areas, together with some distortion or " blistering " of the leaves.

The resistance to ageing *in vitro* is several years, the thermal death point is 90° C. for ten minutes, and it resists 60 per cent. alcohol for one day.

Tobacco Virus 2. Speckled tobacco mosaic. (Type: J. Johnson, *Phytopath.*, 16, p. 141, 1926.) The symptoms on tobacco are mottling and speckling; on petunia, mottling, stunting, malformation and necrosis; on henbane (*Hyoscyamus niger*), chlorosis and malformation. There are no symptoms on *Nicotiana glutinosa*, pepper or pokeweed. The resistance to ageing *in vitro* is three or more months, and the thermal death-point is 90° C. for ten minutes. It withstands 50 per cent. alcohol for one day.

Tobacco Virus 3. Mild tobacco mosaic. (Type: J. Johnson, *Phytopath.*, 16, p. 141, 1926.) The symptoms on tobacco consist of a very mild mottling, which is so readily masked that it may appear and disappear over short periods of time; on *Physalis pubescens* it produces marked stunting and chlorosis; on *Nicotiana glutinosa,* mild chlorosis and stunting; on *N. rustica,* mild mottling and malformation and on *N. glauca* and pokeweed, no symptoms. The resistance to ageing *in vitro* is about six days, the thermal death point is 50° C. for ten minutes, and it is inactivated by 50 per cent. alcohol in one hour.

Tobacco Virus 4. Spot necrosis. A potato virus complex on tobacco, probably the same as the writer's potato viruses X and Y.

Tobacco Virus 5. Ringspot. Johnson's ringspot is a potato virus probably the same as the writer's potato virus X, and is not the tobacco ringspot of Priode (72), Wingard (99), Price (71) and others.

Tobacco Virus 6. Yellow tobacco mosaic. This virus is probably identical with that causing " aucuba " mosaic of the tomato in England (see p. 376).

Tobacco Virus 7. Medium tobacco mosaic. (Type: J. Johnson, *Wisc. Agric. Exp. Stat. Res. Bull.* 76, 1927.) On tobacco the symptoms are medium mottling and stunting; on *N. glauca* and *Petunia,* mild mottling. The resistance to ageing *in vitro* is three months or more, the thermal death-point is 90° C. for ten minutes, and it is not inactivated by 60 per cent. alcohol for one day.

Tobacco Virus 8. Bleaching mosaic. (Type: J. Johnson, *Wisc. Agric. Exp. Stat. Res. Bull.* 76, 1927.) On tobacco the symptoms are mottling with occasional chlorosis on young plants; on *N. glutinosa,* mottling and malformation; on pokeweed, mottling and occasional chlorosis and on *Physalis pubescens,* chlorosis, necrosis and mottling. The resistance to ageing *in vitro* is about three days, the thermal death-point is 90° C. for ten minutes, and the virus is not inactivated by 60 per cent. alcohol in one day.

Tobacco Virus 9. Tomato stem necrosis. This is probably identical with one of the viruses affecting the tomato in England, possibly that known as " stripe " (see p. 373).

What seems to be an additional tobacco mosaic virus has been recorded in Kentucky and is described as " white mosaic " (43). Tobacco plants affected with this virus assume a creamy-white colour in irregular areas between the veins. Narrow bands of blister-like tissue may develop along the veins or an occasional raised blister may appear between the veins. The primary symptom of this disease is the development of circular yellow spots $\frac{1}{16}$ to $\frac{1}{8}$ inch in diameter on the inoculated leaves. E. M. Johnson (loc. cit.) has also described in an extensive monograph other viruses or virus diseases of tobacco, but it cannot be said definitely how these are related to the viruses described by J. Johnson or whether they are single entities or virus mixtures. One of these is " ring mosaic " characterized by definite rings consisting of alternate bands of pale green or yellow tissue. This appears to be a different virus from tobacco ringspot (see below). Another virus described by E. M. Johnson has been named " etch " ; this produces small chlorotic spots on the leaves of the growing point, and a fine necrotic etching on the upper surfaces of the leaves. There is no information on the insect vectors or physical properties of these latter viruses.

Tobacco Ringspot. This disease was first described in America by Fromme, Wingard and Priode (27), and was later studied in greater detail by Wingard (99) and Henderson and Wingard (35). It should not be confused with the " tobacco ringspot " of J. Johnson, nor the X potato virus of the writer, who called this latter virus tobacco ringspot in his earlier papers on the subject. Although the symptoms produced by Wingard's ringspot virus upon tobacco and other hosts closely resemble those induced by the virus of tomato spotted wilt (see under tomato), a comparison of the physical properties of the two viruses shows at once that they are in reality distinct. Infection on the leaf with tobacco ringspot usually results in the development of rings and lines that spread out in a zigzag manner, and these are the most characteristic symptoms of the disease. An initial spot develops on an inoculated leaf in forty-eight to sixty hours, while systemic infection follows about six days after this. The primary rings are usually not more than 1 mm. in diameter, and may appear as small dots, the secondary rings, 3 to 4 mm. in diameter, appear in four days, followed about two days later by a third ring, which is 6 to 7 mm. in diameter. The outlines of the ringspot vary according to the situation. They are circular when occurring in interveinal tissue, but when centred on the larger veins they become very irregular in outline. This virus appears to have a very wide host range, and Wingard records it as affecting plants in seventy-two genera. It is sap-transmissible, but the insect vectors do not appear to be known. Henderson (34) states that the virus is seed-borne when the *Petunia* is the host, but not apparently so transmissible with any other host plants. It is filterable through a Berkfeld *W* candle and remains viable in expressed juice for twenty-two months; both these properties are in marked contrast to the behaviour of the virus of tomato spotted wilt, which symptomatically it much resembles on certain hosts.

Tobacco Leaf Curl. (" Kroepoek " disease in Java : " crinkly dwarf " in South Africa ; " cabbaging " in Nyasaland ; " frenching " in Rhodesia.) An important feature for diagnosis of tobacco leaf curl is the greening and thickening of the smaller veins on the lower surface of the leaf. An extreme form of this vein-thickening is the development of leafy outgrowths or enations. On some varieties a downward curling and savoying of the leaves occurs ; this curling varies considerably in degree (91). Thung identifies three types of " kroepoek " disease, of which two are described by Kerling (46). They are : *Type A*, Common Kroepoek. Symptoms consist of curving of the veins with swellings and cup-like outgrowths. The swellings are round, elongated or irregular nobs. The veins may be thickened and cord-like. In extreme cases the cup-like outgrowths have a stalk about 1 cm. in length supporting a pocket-like lamina of leaf form.

Type B. Curl disease. The leaves, especially the top ones, have regularly downward-curled margins, while the mesophyll between the veins protrudes convexly upwards. The main and lateral veins of the first order are not contorted, and the thickenings of the veins are more regularly spread over the whole lamina.

Type C. Clear Kroepoek. The margins of the leaf are turned upwards, main and lateral veins shorter than normal with small thickenings. Some tertiary veins appear transparent. Thickening of the veins and secondary leaflets does not occur.

The viruses of tobacco leaf curl and the kroepoek diseases are not sap-transmissible and the insect vector is the Aleyrodid *Bemisia gossypiperda*, and possibly an allied species. There is slight evidence that the virus is seed-transmitted as symptoms suggestive of the disease have been noted in England, in tobacco plants grown from the seed of infected plants sent from Rhodesia.

Lycopersicum esculentum (Tomato). *Tomato Stripe* (Fig. 60). This disease was considered at one time to be caused by *Bacillus lathyri*, but this view is no longer tenable. There is no doubt that tomato stripe is a virus disease possibly of a dual nature, of which one virus is of the tobacco mosaic type. The disease has been called tomato streak and " glasshouse streak " by Henderson Smith and Jarrett (42A), but it differs from the American tomato streak (see p. 379), which is due to a tobacco mosaic virus and a potato mosaic virus acting together. The characteristic symptoms of tomato stripe are those occurring on the stem in the form of dark longitudinal streaks or stripes. When a badly affected stem is split open, brown areas may be seen in the tissues of the pith and cortex. The leaves also may be affected and show a mottling of dark and light green similar to that caused by tomato mosaic. On the fruits of affected plants, rounded or irregular sunken blotches sometimes occur, varying in size and of a light or dark brown colour. The virus, or one of the constituent viruses, is easily filterable through the fine grades of Pasteur-Chamberland candles and in undulated sap at a pH of 5·8–6 passes a collodion membrane of average pore diameter 0·26 μ. It is sap-transmissible and there is no exact information on the insect vectors. It has considerable resistance to ageing *in vitro* and to certain chemicals.

As regards differential hosts, the stripe virus, or this constituent of the disease, produces a rather mild light and dark green mottle on tobacco without stem necrosis. It usually becomes systemic with severe stem lesions in *Nicotiana glutinosa* and produces local lesions without systemic infection in *Solanum capsicastrum*. In

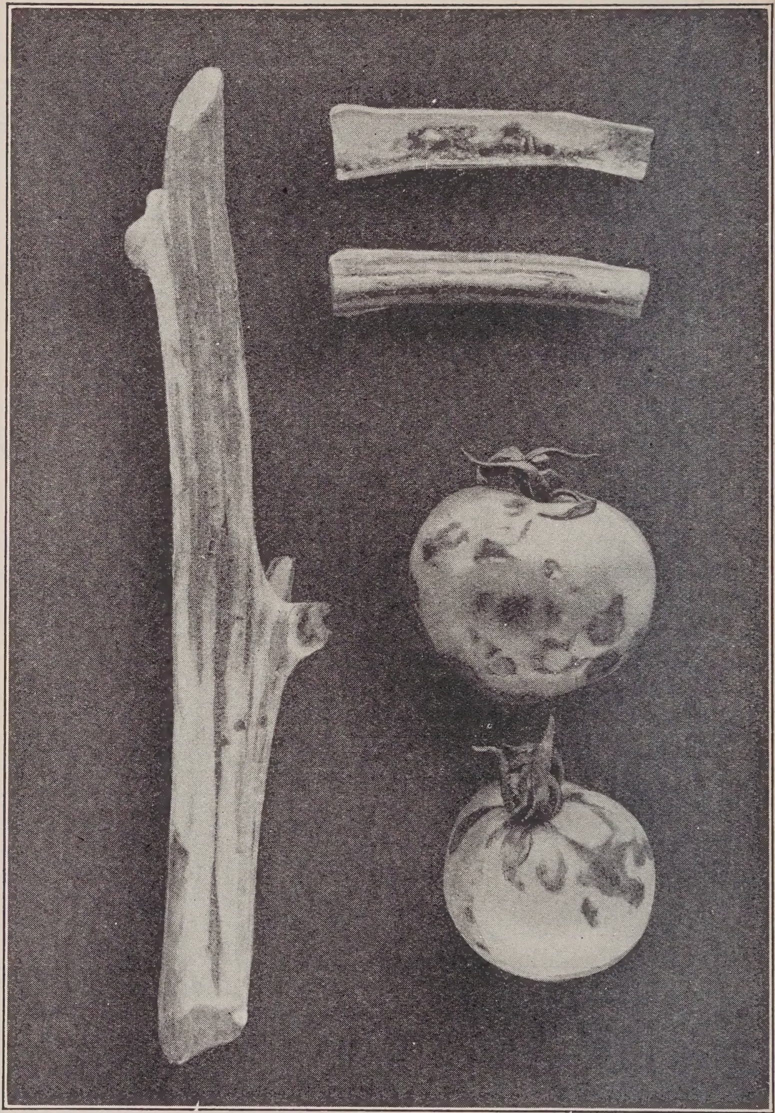

FIG. 60.—Stripe disease of tomato. On the left, portion of an affected tomato stem showing the dark, longitudinal streaks. On the right, above, a short piece of stem cut open lengthwise ; the upper portion shows the browned, shrunken, internal tissues ; the lower, the external streaks. Below, two affected fruits. (After Bewley and Paine.)

many ways this virus closely resembles that of tomato mosaic (see below).

The writer has produced experimentally in tomato a disease which is symptomatically identical with tomato stripe by inoculating the plants with a virus complex consisting of a tomato mosaic virus and the virus of tomato spotted wilt. Although it cannot yet be definitely stated that tomato stripe as it occurs in glasshouses is this virus complex, yet the evidence tends to point in that direction. On several occasions also the writer has isolated these two viruses from tomato plants which had been sent to him as being affected with typical " stripe."

If the above suggestion be correct, it will afford an explanation for the frequent failure to transmit tomato stripe as such to healthy tomato plants by inoculation. Attempted transmission of this disease by sap-inoculation to healthy tomato plants frequently produces in the latter only a mosaic disease without " striping " of the stems. If it be remembered that the virus of spotted wilt is much less infectious by needle inoculation than the tomato mosaic virus, it will be seen that the former virus could easily be dropped from the complex on inoculation.

On the other hand, the fact that Bewley (5) was sometimes able to produce " stripe " in tomatoes by inoculation with sap that had passed an L_2 Pasteur-Chamberland candle is against this theory as the virus of tomato spotted wilt does not pass such a candle.

Tomato Mosaic. The following facts are derived from experiments by the present writer with a mosaic virus commonly found in commercial tomato culture. The symptoms on the tomato take the form of a mild light and dark green mottle without necrosis of leaf or stem. On tobacco dark brown local lesions develop on the rubbed leaf; these coalesce and a progressive necrosis spreads up the petiole to the main stem of the plant on which it produces gross blackened lesions or stripes. These usually prove fatal and the plant dies of a rapid wilt without development of further symptoms. Should this necrosis not prove fatal, secondary symptoms of a mild mottle develop on the leaves of the tobacco plant indistinguishable from that produced by the virus of tomato stripe. It will be seen that this mosaic virus can be differentiated from that of " stripe," which it otherwise closely resembles, by its production of streaks on the tobacco plant and mottle on the tomato instead of the reverse procedure in the case of " stripe." Other characters of the two viruses are similar. Ainsworth, however, considers tomato stripe, or glasshouse streak, to be due to a single virus identical with the tomato mosaic above described (Exp. and Res. Stat., Cheshunt, 1932, 18th Ann. Rept., pp. 42–45).

Tomato Mosaic (Ordinary or mild). A specimen of this virus was kindly given to the writer by Mr. Ainsworth, of Cheshunt. In this case the mottling and leaf deformity on both tomato and tobacco are more pronounced and in symptom-expression and general behaviour the virus closely resembles that of " ordinary " tobacco mosaic (Johnson, No. 1), with which it is probably identical.

Tomato Aucuba Mosaic, Yellow Mosaic (probably James Johnson's tobacco virus 6). This disease was named " aucuba mosaic " by Bewley, who first found it on the tomato in England. First signs of the disease appear in the young developing leaves of the crown about the fifth day after inoculation. These show a downward curling of the whole leaf, with slight turning down of the margins ; the surface of the leaf is rough, wrinkled or corrugated. By the seventh or eighth day points of chlorosis appear on these curled leaves, which rapidly increase in number and tend to coalesce. In severe cases almost the whole leaf surface is pale yellow or white with here and there small islets of intense dark green, which stand up as small blisters. The surface is uneven and there may be turning down of the tip and margins. There is no necrosis and little tendency to extreme malformation, though " fern leaf " has been noted on slowly growing plants. The fruit may show no symptoms or it may be mottled. The virus is sap-transmissible, but the insect vectors are not known. It will stand dilutions up to 1/10,000 and retains its activity for a year or more at room temperature ; it withstands heating for ten minutes at 80° C., but is inactivated at 90° C. It is not inactivated by alcohol up to 90 per cent. (37). (See Fig. 61.)

Tomato Spotted Wilt. This name was given by Brittlebank (11) to a new tomato disease first observed near Melbourne, Australia, in 1915. The initial signs of the disease appear as a slight intensification of the veins of the younger leaves, sometimes

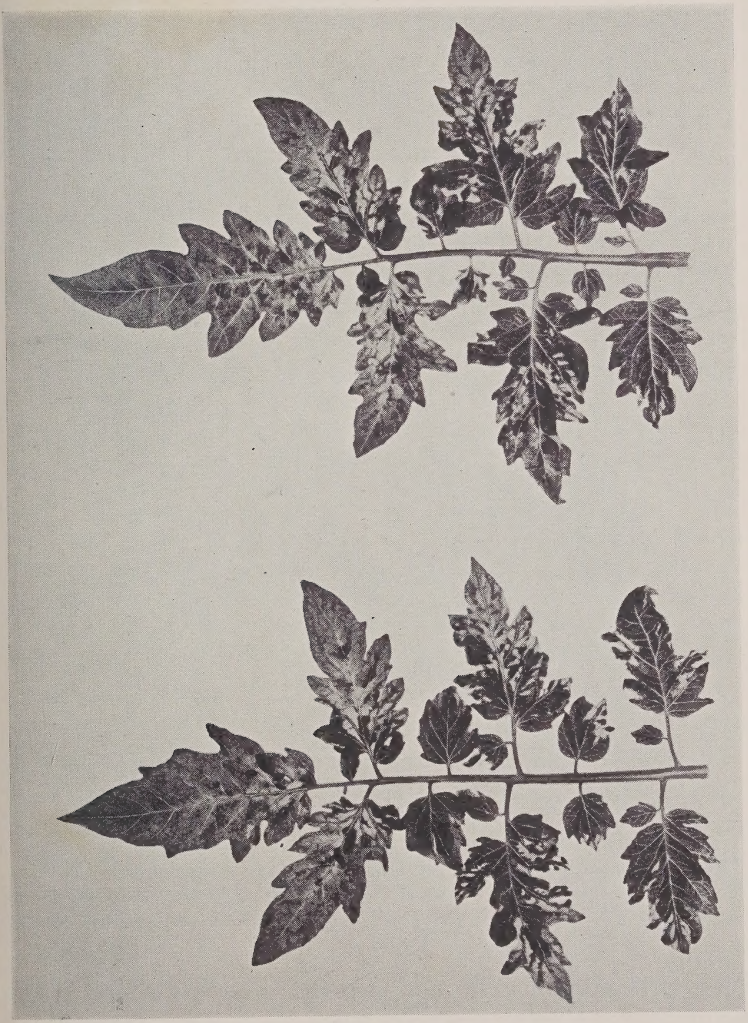

FIG. 61.—Typical leaves of tomato infected with aucuba or yellow mosaic. (After Henderson Smith.)

accompanied by one or two concentric rings. At about the same time the young leaves exhibit a tendency to curl downwards and inwards. The next symptom is the appearance on the leaves of numerous bronze-coloured markings that may be either in the form of isolated circular spots or coalesced into a general bronzing of the whole leaf surface. This latter expression is more common in affected plants out of doors. The bronzing is the most

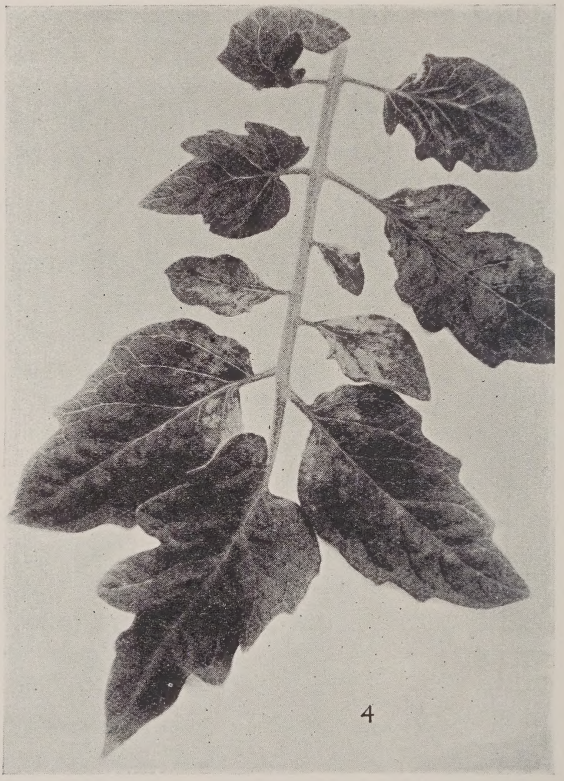

Fig. 62.—Spotted wilt of tomato. Secondary symptoms of the disease; the leaves show, towards their base, a marked mosaic mottling with some distortion. For the early symptoms of this disease, see frontispiece.

characteristic of all the signs of the spotted wilt virus on the tomato (see Frontispiece). Growth of the plant is arrested and it appears stunted beside normal plants. At a later stage of the disease, symptoms take the form of a fairly bold yellowish mottling of the leaves together with some leaf distortion (see Fig. 62). The fruits may show concentric rings or they may exhibit no visible symptoms. It has been found from experiments with this virus upon plant hosts other than the tomato that the predominant tendency of the symptoms is towards the formation of numerous concentric rings (see Fig. 63). The virus is sap-transmissible, more particularly by a gentle rubbing, and the insect vectors are two species of thrips, *Frankliniella insularis* in Australia (3) and *Thrips tabaci* L. in England (83). The virus of spotted wilt has a wide host range and is probably only second in this respect to the viruses of aster yellows and tobacco ringspot. It has been transmitted to various members of the Solanaceæ, Leguminosæ, Compositæ, Campanulaceæ and Tropaeolaceæ. The virus is rapidly inactivated by ageing in expressed juice; it only remains viable for three to four hours at room temperature. Keeping the extracted virus sap at low temperatures, such as — 10° C. seems to have no effect in prolonging the viability of the virus. It is not filterable through Pasteur-Chamberland or Berkfeld candles, but the writer has succeeded in filtering it through a "Gradocol" collodion membrane of average pore diameter of 0·45 μ. The thermal death-point is low, about 42° C. for ten minutes. Spotted wilt is of common occurrence in Australia, where it is of considerable economic importance, and the disease is widespread in the British Isles (84), it has also been recorded from the United States of America (19).

Tomato Streak (American). This is a composite virus disease consisting usually of a potato mosaic virus and a virus of the tobacco mosaic type. It should not be confused with the English tomato stripe, also sometimes called streak, which may or may not be due to a single virus. The symptoms of American tomato streak as described by Vanterpool (95) consist of necrotic streaks on the stems and spots on the leaves. These spots, which are irregular in colour, subsequently turn brown and are surrounded by yellow areas. Complete wilting of the leaves, accompanied by necrosis and drying out, may occur. At a later stage of the

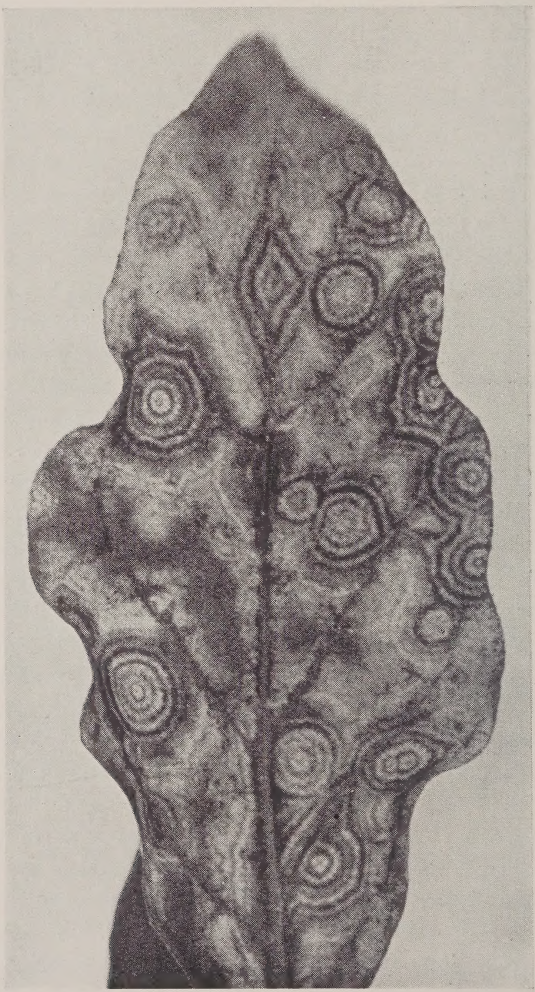

Fig. 63.—The virus of tomato spotted wilt on *Solanum capsicastrum*. On this plant the symptoms take the form of numerous concentric rings and diamond-shaped patterns.

disease the upper growth becomes spindling and distorted, the leaves are small and ruffled, weak, crinkled and curled downwards with numerous yellowed and minute necrotic areas. At this stage marked mosaic symptoms are invariably present. The brown linear lesions on the stem vary from 1 mm. to several cms. in length. They enlarge and sometimes coalesce. The lesions on the petiole are often so large as to cause the leaf to shrivel and dry out. The fruit may be entirely free from outward blemishes. Tomato streak can be produced by the admixture of potato virus X with a number of tomato and tobacco mosaic viruses. Thus the viruses of mild tomato mosaic, tomato stripe, tomato aucuba mosaic, a yellow mosaic of tobacco and all the tobacco mosaic viruses tested by the writer, when mixed with potato virus X, give typical streak on tomato. Admixture of potato virus Y with any of the foregoing viruses fails to produce tomato streak. Valleau and Johnson (94) give a list of the virus combinations which produce tomato streak in America.

Bunchy Top of Tomato. This disease has been recorded and described for the first time in South Africa (55). The first indication of the disease is a sudden and almost total cessation of growth at the branch extremities, with the result that at these points the leaves become closely crowded, giving to the plant the bunched appearance which is typical of the early stages of infection. The most characteristic modifications are severe stunting of the entire plant, in particular the production of small leaves, a necrosis of the leaves and stems, and various forms of leaflet distortion, such as curling and an abnormal unevenness of the surface. Chlorosis and mottling are absent in this disease. Necrosis is characteristic of the early stages of infection and narrow brown or black streaks appear along the under surface of the rhachis and leaflet veins a few days after the appearance of the first signs of the disease. This is accompanied or followed by broad black streaks along both surfaces of the rhachis and by the blackening of leaflet veins for a varying part of their length. Fruits from affected plants are small, sometimes distorted and of no commercial value. They are either seedless or contain a few small seeds, of which only a few are fertile. The virus of tomato bunchy top is sap-transmissible but not apparently filterable by candle methods. The insect vectors are not known.

The tomato plant is susceptible to a tobacco mosaic virus which produces a bright yellow mottling of the foliage somewhat similar to "aucuba" mosaic but brighter in colour. The virus of curly top of sugar beet (see next section) also attacks the tomato, the disease it produces being known as "western yellow blight" (26).

Chenopodiaceæ

Sugar Beet. *Curly top* (*curly leaf*). When a plant is infected with the virus of curly top, the mature leaves remain normal in appearance while swelling of the veins and curling of the leaves develop on the new growth. There is no conspicuous colour change in the early stages of the disease. In the later stages the older leaves, which were mature at the time of infection, turn yellow and die, while the younger central leaves, which are dwarfed and usually show conspicuous vein swellings on the under sides, remain green. On the whole, diseased leaves are dark green, brittle and thicker than normal. The vein swellings vary with different individual plants from cases where practically every veinlet is irregularly swollen to cases which show only scattered, inconspicuous, rounded swellings. The disease is characterized by a necrosis of the phloem throughout the vascular system and in the advanced stages of the disease this necrosis shows in the roots as dark concentric rings. The exudation of a clear viscous liquid is often conspicuous on the oldest leaves which show the vein swellings. The virus of curly top of sugar beet is sap-transmissible only with difficulty, and the specific insect vector is the leaf hopper *Eutettix tenellus* Baker. Recently, in the Argentine, what is apparently the same virus has been transmitted by another species of leaf hopper, *Agallia sticticollis* Stål. (26).

Curly top virus is filterable through the three grades of Berkfeld candles (82). The host range of this virus is very wide and members of the following families have been found naturally infected or have been infected experimentally : Chenopodiaceæ, Leguminosæ, Cucurbitaceæ, Cruciferæ, Umbelliferæ, Solanaceæ, Polygonaceæ, Malvaceæ, Linaceæ, Boraginaceæ and Valerianaceæ (81). Curly top appears to be confined to North and South America and is especially prevalent in California.

Mosaic of Sugar Beet. In this disease the mottling of the leaves is characteristic and is produced by the occurrence of light green areas in the foliage. These pale areas often take the form of a ring round darker tissue. In the youngest leaves the pallid tissue may predominate, whereas in older leaves the normal dark green usually predominates. Malformation may or may not be associated with mottling. As a rule, a young leaf with marked mottling shows puckering and crinkling of the mesophyll. A characteristic malformation is the bending back of the leaf near the tip, curling and crinkling of the leaf margin and tip, frequently followed by death of the tip. The virus also affects the garden beet, on which it produces a similar mottling and leaf distortion. The insect vectors appear to be the aphides *Myzus persicæ* Sulz., *Macrosiphum gei* Theob. and possibly *Aphis rumicis* L. (=*fabæ Scop.*).

According to Hoggan (39) systemic infection becomes apparent in the form of minute yellow or light green flecks on the young leaves, five or more days after infection. These increase in size, the mosaic pattern soon becoming resolved into a sharply defined, fine speckly mottling quite different from the chlorosis or coarse indefinite mottling characteristic of the virus of cucumber mosaic on this host. Leaves of later development usually show a similar type of mottling. The virus of sugar beet mosaic is sap-transmissible and can be transferred to healthy plants and to spinach by rather hard rubbing of the leaves. It does not appear to be seed-borne (see also 6, 45, 73). Mosaic of sugar beet is common in the U.S.A. and in Germany and appears to be spreading in the British Isles.

Leaf Curl or Leaf Crinkle of Sugar Beet. A serious virus disease of the sugar beet, similar to but apparently distinct from curly top, has been described in Germany. There are said to be both primary and secondary symptoms, the development of the latter occurring after an incubation period of varying duration. Primary symptoms consist of pale spots, reduction of turgor and occasionally premature death. The secondary symptoms include swelling, glassiness and distortion of the leaf veins and petioles. The leaf parenchyma is inflated, with consequent curling, curtailment and inward bending of the petioles, which gives a lettuce-head appearance to the plants. Growth is arrested and the older leaves wither. There is a conical projection of the growing point and numerous small lateral roots form on both sides of the main one ; the plants die prematurely. The insect vector is a lace bug, *Zosmenus* (*Piesma*) *quadrata* Fieb. (98).

The sugar beet is also susceptible to the virus of cucumber mosaic (= spinach blight). According to Hoggan (39) no systemic infection was secured by artificial inoculation (*i.e.*, rubbing). No systemic infection was produced when infective aphides were confined to a single leaf. Systemic infection was produced, however, if infective aphides were allowed to wander over the plant.

Spinach (*Spinach Blight*). This virus disease is of the mosaic type and is characterized by mottling and malformation of the leaves, the affected plants being eventually killed. The plant first develops a pronounced yellowing on the younger leaves which is followed by malformation. The plants are stunted in growth. A later stage of the disease is a development of necrosis and browning of the tissue. The younger leaves and crowns continue to live for some time after the older leaves are killed, but death of the whole plant finally ensues.

The virus of spinach blight is transmissible by needle inoculation and the insect vectors are the aphides *Myzus persicæ* Sulz., *Macrosiphum gei* Theob. and *Aphis rumicis* L. Possibly the capsid bug *Lygus pratensis* is also concerned in its transmission (56). Hoggan (39) has shown that the virus of spinach blight is the same entity as the virus of American cucumber mosaic.

Spinach Mosaic. This disease is caused by the virus of sugar beet mosaic (39) and what is probably the same disease of spinach is known in Germany as *Mosaikkrankheit* or *Gelbfleckigkeit.* The first sign of infection is a marked downward arching of the young leaves, accompanied or soon followed by the appearance of numerous light golden yellow flecks on the young leaves. There is no faint uniform chlorosis of the foliage as in spinach " blight," but a distinct spotting. Leaves of later development show similar symptoms with some dwarfing and puckering, but there is no such marked malformation as is characteristic of spinach blight. As the disease progresses, the yellow coloration gradually spreads over the whole of the foliage, the plant becomes stunted and growth ceases. At this stage the disease resembles closely the symptoms of spinach blight.

Polygonaceæ

Rumex spp. (the Dock). Broad-leaved dock (*Rumex obtusifolius*) is susceptible to a mosaic disease which produces a mottle chlorosis of the younger foliage. There is no marked stunting or other deviation from normal habit. The mottling assumes two distinct forms, occurring in some plants in the interveinal spaces, while in others large patches extend over several veinlets. *Rumex lanceolatus* is also susceptible to this disease. The virus is sap-transmissible, but is not, apparently, filterable through various candles (31).

Böning (6) has recently described a virus disease affecting *Rumex obtusifolius*, *R. crispus*, beets and spinach, in which the chief symptoms are reduction of the leaf surface, thickening and extreme brittleness of the tissues. Sometimes the midrib is all that remains of the leaf. In other cases the blades show a downward rolling accompanied by irregular malformation. This virus is probably distinct from the other viruses affecting similar plants. The insect vectors are thought to be the aphides *Myzus persicæ* and *Aphis rumicis*.

Santalaceæ

Santalum album L. (Sandal Wood). This parasitic tree is affected by a virus disease known as *spike*, which causes severe losses to the sandal-wood industry in India. The disease appears to be of the rosette type and the growth of "spiked" and healthy buds is entirely different. The healthy leaves are broad and light green in colour, the internodes are long and there is no tendency to branch off laterally. The growth of the spiked bud is much slower and is characteristic. The leaves are small and deep green in colour ; the internodes are very short and the branching is very marked, imparting a typical bushy appearance to the entire growth. The first signs of the disease are generally seen in solitary twigs or branches, while the other parts remain healthy, but occasionally the whole tree may show the symptoms all at once. Spiked twigs do not bear flowers, but the healthy parts of a partially spiked tree flower profusely and give rise to healthy seedlings. The virus is transmissible by bark-graft or by leaf-insertion but not by sap-inoculation. The insect vectors are not known. The disease seems to be widely spread in the sandal areas of Coorg, Mysore and Madras. The whole problem is under intensive survey by the Indian Institute of Science at Bangalore in co-operation with the Forest Research Institute, Dehra Dun. The reader is referred to numerous papers which have appeared in the *Journal of the Indian Institute of Science*, Vols. 11A, 12A, 13A, 14A, and to a *résumé* of entomological investigations by Dover (22).

Urticaceæ

The Hop. There appear to be three virus diseases which affect the hop plant : these are *nettle-head*, *mosaic*, and one, recently described, known as the *chlorotic disease*.

Nettle-head stunts the affected plant and renders it sterile, or nearly so, but does not kill it. The bines have a weak growth with small leaves and shortened internodes. The disease does not appear to spread very rapidly. The virus is graft-transmissible, but the insect vectors are not certainly known, though Blattný has stated that he has succeeded in transmitting a similar disease of hops in Czecho-Slovakia by means of the leaf hopper *Chlorita flavescens* F.

Mosaic of hops is a more lethal and widely spread disease. The characteristic symptoms are mottled green and yellow areas in the leaves with recurving of the edges, and a noticeable brittleness of the leaves and petioles and of the tips of the bines. The yellow areas are translucent and are clearly visible when viewed against the light. The first symptoms are the development of mottling and an abnormal shape of the newer leaves of the laterals. In the season following infection, the bines fail to grow to their normal length, fall away from their supports and die back at the tips, producing few or no hops (93). The virus of hop mosaic is transmissible by grafting, but not, apparently, by inoculation. The insect vectors of the virus of hop mosaic are not known (see also 76, 77).

Chlorotic Disease. This disease has only recently been described (78). In a hop plant which has already made growth, some or all of the primary leaves exhibit on the lamina areas of pale yellow or pale greenish yellow. When the leaf is viewed against the light, the colour of the affected parts is primrose yellow. This abnormal coloration may cover only a small part of the lamina or it may invade practically the whole surface. Leaves with large chlorotic areas are very commonly distorted, the green parts continuing growth and developing into domed or bulbous parts. Lack of growth at the chlorotic margins serves to increase the distortion, and parti-coloured leaves, closely down-curled, are a frequent feature of the disease. Histological examination of chlorotic leaves shows a great reduction in the number of chloroplasts in the cells of the yellow parts of the lamina. The virus is transmissible by budding and grafting and also by rubbing healthy leaves with virus sap. Symptoms do not develop until the year following that in which the inoculations were made. The insect vectors are not known (see Fig. 64).

Ficus carica (the Fig). A mosaic-like trouble of fig leaves has long been recognized in California. It may almost be said that every fig tree in the State shows traces of mosaic, although the disease is not generally regarded as commercially important. Areas of lighter tissue occur and leaves may be distorted. Both leaves and fruits are affected and in severe cases may drop. Studies are reported on the comparative susceptibility of varieties. Aside from *Blastophaga* spp., *Blastophaga psenes* and visitors to the ripe fruit, the insect population on figs is small. Aphides and thrips are present for brief periods, while the fig mite, *Eriophyes*

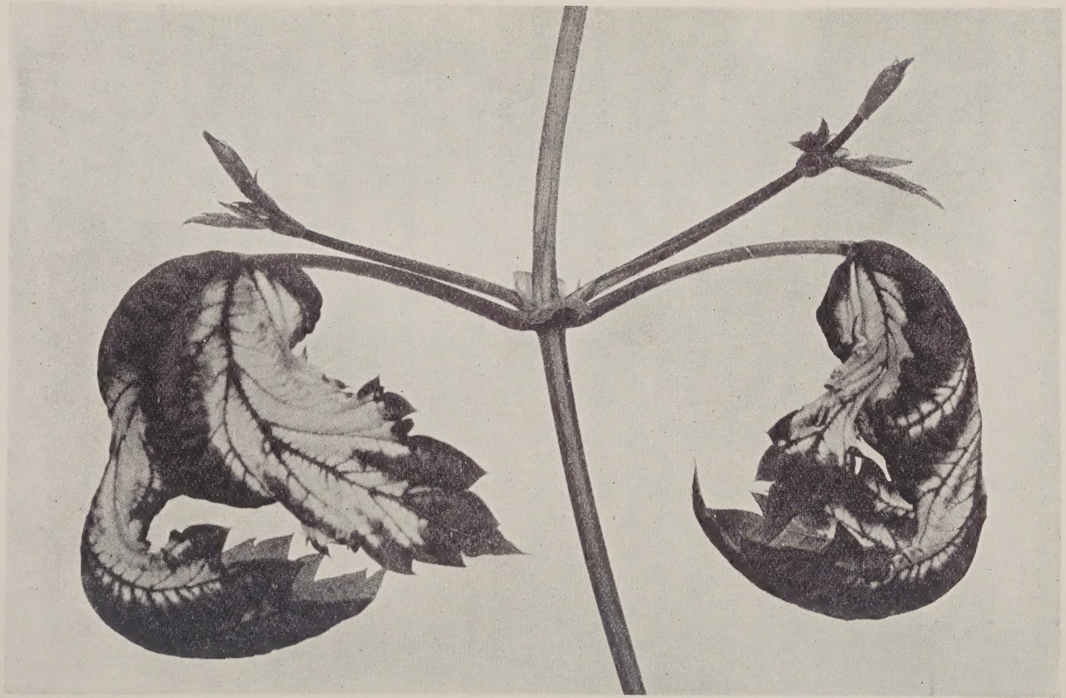

FIG. 64.—The chlorotic disease of the hop. The light-coloured parts of the leaf are lacking in chloroplasts. Chlorosis at the margins has caused restriction of growth and consequent curvature of the lamina. (After Salmon and Ware.)

ficii, is abundant and widely distributed. A mosaic vector has not been found.

Cuttings from mosaic-affected trees propagated in the greenhouse continue to show mosaic.

Seedlings from affected plants have remained healthy for about a year in the greenhouse, whereas buds from healthy seedlings set in diseased trees have grown into mosaic-affected shoots.

Seedlings several years in the orchard show much mosaic. The trouble under consideration is apparently a true virus disease (13).

MONOCOTYLEDONS

Iridaceæ

Iris. A mosaic virus affects this plant and is definitely on the increase in bulbous Iris, and it also apparently attacks the rhizomatous Iris (*Iris tuberosa*). Mosaic in bulbous Iris has been observed in English Iris (*I. xiphoides*), Spanish Iris (*I. xiphium*), Morocco Iris (*I. tingitana*) and Dutch Iris (*I. xiphium hybridum*). Judging from experience of commercial stocks it is most general in varieties produced from crosses obtained from *Tingitana*—*e.g.*, Wedgewood, the most popular commercial variety. In this variety mosaic was uncommon until three years ago. In 1933 the majority of commercial stocks of Wedgewood show from 30 to 80 per cent. infection. As opposed to this rapid increase the variety Juliana may be mentioned. Bulbs of Juliana are usually imported from Southern France, and for many years a percentage, which remains fairly constant from year to year, have shown typical mosaic symptoms. The most definite symptoms are longitudinal streaks of yellow in the foliage and the flower stems, which are strictly comparable to the streaks produced in Yellow Stripe of Narcissus (see Amaryllidaceæ). Contrary to general expectation, exposure to high temperatures does not appear to mask the symptoms. Infected plants produce definitely flabby and somewhat shorter flower stems than healthy bulbs (Hodson *in litt.*). The insect vectors of the virus of Iris mosaic are not known, though the present writer has slight evidence that the aphis *Macrosiphum gei* may be concerned in its dissemination.

Gladiolus (*Mosaic*). The corms of Gladiolus affected with mosaic are warty instead of smooth and mottled in colour. Leaves from affected corms only grow to about half normal length. They are thicker and stiffer than normal leaves and have a mottled appearance. The flowers of affected plants exhibit " breaking " of the colour in a marked degree. This virus may be the same as that causing " breaking " of tulips (see Liliaceæ), and it is thought to be aphis-transmitted (21).

Amaryllidaceæ

Narcissus (*Yellow Stripe*, *Mosaic*, *Grey Disease*). Narcissus plants affected with *yellow stripe*, or " grey disease " as it is called in America, exhibit a streaking or striping of the leaf and a hyaline streaking of the flowers. Hodson (*in litt.*) states that three main types of symptom are recognizable, but they tend to grade, one into the other. Each type shows as alternating streaks of normal green and lighter green and/or yellowish tissues and may be classified as follows :—

(1) *Sir Watkin Stripe.* A greater or lesser amount of mottling present, no leaf distortion ; there is some evidence that bulbs tend to become reduced in size and vigour. Many commercial varieties are affected, notably Sir Watkin, Golden Spur and Lucifer.

(2) *Talma Stripe.* Similar to the foregoing, but in addition, definite corrugation and roughening of the leaf surface are present. It is almost impossible to find a stock of the variety Minister Talma free from stripe. The disease is also common in the variety Czarina.

(3) *Alfred Stripe.* This type is more virulent than the other two, the chlorotic areas are bright yellow in colour, the foliage is severely distorted and infected bulbs have no commercial value. This type of symptom is common in King Alfred, Victoria and certain other varieties.

The virus of *grey disease* in America, which is thought to be identical with that of the English disease, *yellow stripe*, is transmissible by needle inoculation, and it is further suggested that the disease is spread through root contact, especially of injured roots. McWhorter (62), in America, also finds very great varietal symptom variation. Symptoms accountable to chlorophyll repression include yellow striping, mottling, marbling and, occasionally, yellowing of entire leaves. The leaf surfaces of some varieties become roughened, in others the leaves may curl or bend in the plane of the leaf.

The insect vectors are not known, and Hodson (*in litt.*) considers that available evidence is against the possibility of *Thrips tabaci* being the vector. He suggests the mite, *Tarsonemus affraximatus* var. *narcissi* Banks, as being worthy of consideration in this respect. Seed from diseased narcissi produce healthy seedlings and the virus is not inactivated by immersion of affected bulbs in water at 110° F. for three hours (38, 63).

Agave rigida var. **sisalana** (Sisal Hemp). A new virus disease of sisal in the Belgian Congo has been described. Pale green transparent spots, which later turn yellow with brownish red margins, develop on the young leaves of plants from six months to three years old. The insect vectors are not known (86).

Liliaceæ

Allium, Onion (*Yellow Dwarf, Crinkles*). Onions affected with the virus of yellow dwarf or crinkles exhibit yellowing and stunting of the plant, accompanied by drooping and frequently by crinkling and chlorosis of the leaves. Under certain conditions the symptoms may be completely masked until after the bulbs have undergone a rest period and then have been regrown. The virus is not seed-transmitted. The insect vectors are said to be six species of aphides—the black bean aphis (*Aphis rumicis* Linn.), the corn leaf aphis (*Aphis maidis* Fitch), the apple grain aphis (*Rhopalosiphum prunifoliæ* Fitch), the green peach aphis (*Myzus persicæ* Sulz.), the potato aphis (*Macrosiphum gei* Koch) and the melon aphis (*Aphis gossypii*, Glover), and possibly the six-spotted leaf hopper (*Cicadula sexnotata* Fall).

The virus of yellow dwarf becomes inactivated after ageing for

112 hours at 29° C.; the thermal death-point lies between 75°–80° C. when heated for ten minutes. The virus is sap-transmissible (23). Yellow dwarf of onion appears to have been recorded only from the United States of America.

Lilium spp. The two chief virus diseases affecting lilies are *yellow flat* and *mosaic.* The first-named is of the "rosette" type (see peanuts, peach, banana and wheat) and affects the Bermuda Easter lily (*Lilium longiflorum* var. *eximium* Baker). The leaves of diseased plants, especially the upper and youngest leaves, are very markedly curled downwards; they are not much shorter than normal leaves, but reach outward only to about one-half the distance because of their downward curling. In some cases the leaves may be distorted and twist sideways; they do not form a shallow trough as in normal plants, but the upper surfaces are flat or slightly convex in cross section. The colour of the leaves is slightly chlorotic, but there are no streaks or spots. The general appearance of an affected plant is that of a flat rosette or cylinder in contrast to the pyramidal shape of normal plants (see Fig. 65). The virus is not sap-transmissible and the insect vector is the melon aphis (*Aphis gossypii* Glover). There is no evidence that the virus is seed- or soil-transmitted. The disease is prevalent in Bermuda and also in Japan, where it occurs on *Lilium giganteum* and *L. formosum.* It has been recorded in Europe on imported bulbs (66).

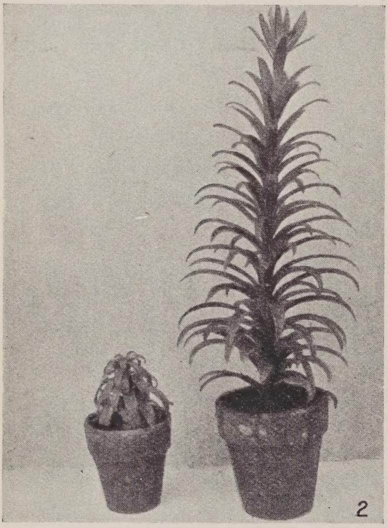

Fig. 65.—Yellow flat or rosette of the Easter lily (*Lilium longiflorum*, var. *eximium* Baker). Normal plant on right. (After Ogilvie.)

Mosaic. In the mosaic disease of the Easter lily there appear to be three main types of symptoms. In the first type the leaves are curled downwards and have chlorotic streaks which later give rise to rusty necrotic areas. The number of flowers is reduced and the flowers themselves are distorted, sometimes showing the chlorotic stripes and mottling characteristic of the diseased leaves. Diseased secondary shoots are apparently always produced. In the second type of symptom expression the disease is in a mild form in which minute, pale green or yellowish linear spots appear on the leaves. The pronounced chlorotic appearance and marked curling of the leaves are absent, though twisting of the leaves is common. In the third type, which is characteristic of the virus on certain strains of *L. longiflorum* var. *eximium*, the leaves have long, tapering points and show distinct mosaic mottling. The flowers are nearly always aborted, the flower parts remaining green and splitting (68).

In *Lilium auratum* and *L. speciosum* the mosaic symptoms are very marked and the effect of the disease is serious. The mottling is much coarser than in *L. longiflorum* and shows itself as elongated yellow streaks and blotches resembling a form of variegation. The " dying off " which is frequently met with in these two species is believed to be due to infection with the mosaic virus.

Mosaic-affected plants of *Lilium candidum* show an indistinct mottling of the leaves and often have a slight yellow cast. The mottling can be more easily seen if a leaf be detached and held up to the light. In summer the symptoms may be partly masked.

The wholesale degeneration of this species of lily is also considered to be due to infection of the stocks with mosaic virus (14).

The insect vector of lily mosaic is *Aphis gossypii* Glov. and the virus appears to be sap-transmissible (33).

Tulipa (*Mosaic*, " *Break* "). The well-known phenomenon of "breaking" in the garden tulip, whereby the flower becomes variegated, is due to a virus of the mosaic type. This is one of the few cases of a plant virus improving a plant from the æsthetic and commercial point of view, as the breaking of the colour produces some attractive variegation. There are two types of colour breaking, the *self* (= red) and the *clear* (= white). The

" self " break consists of irregular areas of concentrated pigment on the original self ground (see Fig. 66). The " clear " break consists of irregular areas of concentrated pigment and of self colour on a ground devoid of anthocyanin. The yellow or white ground colour depends upon the presence or absence of yellow plastids in the mesophyll cells (12). The leaves of affected tulips may also show mottling and irregular striping. The insect vectors in England are the aphides *Myzus persicæ* Sulz. and *Macrosiphum gei* Theob. (57), and in the United States of America these two and possibly *Myzus pelargonii* Kalt.

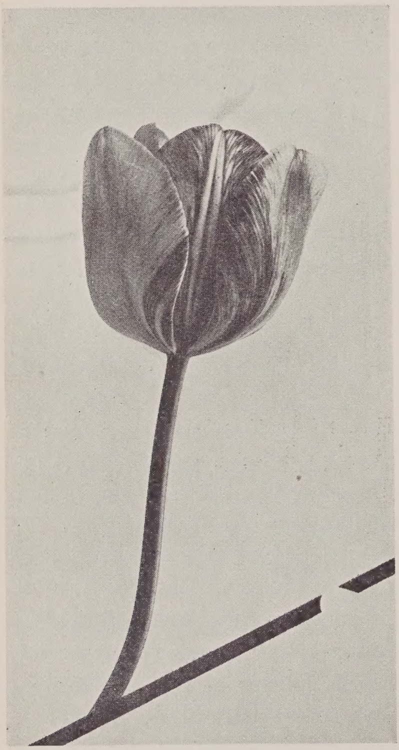

Fig. 66.—" Breaking " in tulips. Red, or self, " break " in tulip Clara Butt. (After McKenny Hughes.)

McWhorter (61) advances the theory that tulip " breaking " results from the action or interaction of two viruses. One of these viruses carries a colour-adding factor and produces no visible effect on the leaves ; the other removes flower colour and strongly stripes the leaves. It was observed that certain varieties, when " broken," tend to segregate into plants or parts of plants (clumps) bearing strongly darkened flowers or decidedly bleached flowers. By encouraging this segregation through selection and by using inoculum prepared from the parts of flowers where the colour was removed and darkened respectively, it has been possible to secure the viruses in almost pure condition. The colour-adding virus has little effect on plant growth and may be of practical use in producing " new " varieties. McWhorter states (*in litt.*) : " The colour-adding virus is so powerful in its action that it is possible to turn a white tulip bright red or a pink tulip vermilion or to make a ' Francombe Sanders ' out of a ' Clara Butt.' " The colour-removing virus is extremely virulent and reduces plant growth to one-third normal. Cross-inoculation with the juice from leaves of mosaic-diseased *speciosum* lily indicates that these contain a virus indistinguishable from, if not identical with, the colour-removing virus of tulips.

Araceæ

Anthurium spp. A virus disease apparently of the mosaic type has recently been described affecting *Anthurium andrœanum* and *A. scherzerianum.* Symptoms take the form of pale yellow lines and spots on the leaves followed by curling and stunting. The virus is transmissible by inoculation, but the insect vectors are not known (96).

Musaceæ

Banana (*Bunchy Top*). The banana is attacked by a serious virus disease of the rosette type, known as " bunchy top." The first external symptom is the appearance of irregular, nodular, dark green streaks along the secondary veins on the underside of the lower portion of the first unfurling leaf. In the following leaf, while still unfurled, pale whitish streaks may develop and the leaf may unfold slightly abnormally. The presence of the characteristic broken dark green streaks along the secondary veins of the lamina, or along the midrib or petiole, is the most definite and reliable symptom of bunchy top. As the disease progresses, the leaves show a certain amount of chlorosis, particularly on the margins of the laminæ, and are generally of a more yellowish hue than normal. The leaves of a bunchy top plant are shortened, narrow, brittle, upright, wavy and slightly rolled. After several abnormal leaves have appeared, extreme congestion is apparent at the apex of the pseudo-stem and the plant assumes a " rosetted " condition. The roots of a bunchy top plant show a much greater tendency to decay than do the roots of healthy plants, and although new, and at first apparently healthy, roots are formed, these produce an unusual number of lateral roots which become purple in colour and decay in their turn. This abnormal tendency of the roots to decay is considered to be secondary and may be due to a loss of immunity induced by the virus to decay-producing organisms. Histological examination reveals changes in the vascular bundles corresponding with the external symptoms of broken dark green streaks in the lamina, midrib and petiole. The changes are as follows: in the phloem region there is a suppression of development of the fibrous sheath. The region in which fibres are normally present is occupied by cells which are heavily laden with chromatophores. The fundamental tissue in the neighbourhood of the phloem is loaded with chromatophores. In the normal plant this region is almost entirely devoid of chlorophyll. "Appositional" growth has taken place in the fundamental cells in contact with the phloem. The original cells have been divided up into angular cells by the growth of thin cellulose walls. Growth of these cells has apparently taken place in all planes. The newly formed cells contain numerous chromatophores and distinct nuclei. "Appositional" growth of cell walls along all planes has taken place in the sieve tubes and companion cells of the phloem, giving rise to numerous angular cells along their length. These cells are uninucleate with nuclei of an abnormal type. The abundance of these nuclei in the phloem of a primary affected plant is a most noticeable feature in any transverse or longitudinal section. The virus of bunchy top is not sap-transmissible and the insect vector is the dark banana aphis *Pentalonia nigronervosa* Coquerel. The host range seems confined to the *Musaceæ*. Bunchy top of bananas made its first appearance in Australia in 1913, and has since spread through the banana areas of north-eastern New South Wales and south-eastern Queensland. It is also prevalent in Fiji, Ceylon and Egypt (52).

See also Darnell-Smith (15) and Goddard (29).

An apparently new virus disease of the mosaic type affecting the banana has recently been described in New South Wales (53).

Affected plants exhibit severe yellowing and mottling of the younger foliage, followed by a rotting of the heart leaf and central portion of the pseudo-stem. The " heart rot " stage of the disease apparently only occurs during the winter months, but the yellowing and mottling may persist throughout the year. In young plants the disease first appears as whitish or yellowish white streaks, which may extend from the midrib to the margin of the most central leaves. These chlorotic areas vary from narrow streaks to bands ½ inch or more in width. In some leaves the streaks are not continuous, giving rise to a mottled appearance of yellow and green areas. During the summer infected plants continue to produce this mottled type of foliage, but with the advent of cooler weather many of the plants commence to rot at the heart leaf, the rotting extending down into the corms and finally killing the plants. The virus is transmitted by the banana aphis *Pentalonia nigronervosa.*

Manila Hemp. Manila hemp is affected with a bunchy top disease due, presumably, to the same virus as that which attacks the banana. The symptoms consist of indefinite chlorotic areas on the margins of the youngest leaves—curling, browning and finally rotting from above downwards. As in the case of the banana, the virus is not sap-transmissible and the vector is the same aphis, *Pentalonia nigronervosa* (65).

Graminaceæ

Saccharum officinarum, Sugar Cane (*Mosaic, yellow stripe,* "*gelestreipensiekte*"). In mosaic of sugar cane, early symptoms are indicated by the appearance of irregular light-coloured streaks or spots on the leaves. Two distinct colour types are observed, one is merely a dilution of the normal colour, while in the second type, yellow is predominant and the affected areas have a decided yellowish green appearance. In the light-coloured areas small white, opaque spots and streaks may develop. There is a sharp demarcation between normal and affected areas. Considerable stunting injury is produced, and in the year following infection, a canker of the stalks may be observed. The aspect of a field of diseased plants is said to resemble the effects of a severe drought with yellowing of the foliage and stunting of the plants (9). The virus of sugar cane mosaic appears to attack a large number of wild grasses such as *Echinochloa colonum* L. and *Syntherisma sanguinalis* L. (97) and the wild cane *Gynerium sagittatum* Beauv. (1). On the other hand, Storey (89) describes a mosaic disease of maize and sorghum in South Africa which is not transmissible to sugar cane. The virus of sugar cane mosaic is sap-inoculable, and the insect vector, which appears to be specific, is *Aphis maidis*. Intracellular bodies have been described as associated with sugar cane mosaic (24).

The sugar cane is susceptible to two other apparent virus diseases : one of these is the " sereh " disease, in which growth is arrested, the majority of shoots remaining short and stunted (51). The other is known as " Fiji " disease, which is characterized by the production of elongated galls in the phloem tissues. The galls are irregularly distributed in both stalks and leaves (47).

The vector of the virus of "Fiji" disease has recently been shown to be a leaf hopper, *Perkinsiella saccharacida* Kirk. (64A).

Oryza sativa (Rice). The rice plant is affected by a virus disease of the mosaic type, usually known as the dwarf or stunt disease. It is characterized by small, elongated, chlorotic areas, or white specks when viewed by transmitted light, arranged along the veins of leaves, and by stunting of the whole plant followed by excessive tillering and a dark green coloration. The white specks, which develop before the leaves unfold, elongate and spread out along the leaf parallel to the midrib, forming fine interrupted streaks. Plants infected in their very early stage of growth become severely stunted and produce only a few small worthless panicles or none at all. The virus is not transmissible by the seed or soil, nor by mechanical methods. The insect vector is the leaf hopper *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl.

Intracellular bodies are associated with the dwarf disease of rice (28).

Wheat, Rye. One or more mosaic viruses attack wheat and rye, causing a " rosette " disease. The mottling consists of irregular streaks, which vary in length and width. In some cases mottled leaves show a light green pattern on the normal green background. In certain varieties the leaves frequently develop light yellow patterns or irregular stripes, while it is not uncommon to find a mosaic mottling on the leaf sheaths and glumes. The green and yellow types of mottling may be caused by one virus or by distinct viruses, but are considered to show characteristics which differ from those manifested by the tropical mosaic occurring on sugar cane, Indian corn and other species belonging largely to the higher tribes of grasses. The virus of wheat mosaic appears to be confined in host range largely to the *Hordeæ*, and it has not been transmitted experimentally by *Aphis maidis* or by other wheat-feeding aphides. There is no evidence that the virus of wheat mosaic is transmitted through the seed, but it is considered to be carried in the soil (58). Intracellular bodies occur in the cells of mosaic-diseased wheat (25).

Maize (*Streak Disease*). The streak disease, when fully developed in the maize plant, is characterized by a pronounced chlorosis of the leaves confined to narrow broken stripes arranged along the veins (see Fig. 67). The stripes vary from a few millimetres to several centimetres in length, and are individually from 0·5 to 1 mm. in width, although frequently fusing laterally to form wider composite stripes. The chlorotic tissue generally appears an opaque yellow when viewed by transmitted light, affording a marked contrast to the deep green of the remainder of the leaf. On some leaves a small proportion of the stripes may appear translucent, and in this case the tissue may be slightly shrunken. The stripes are nearly evenly distributed over every leaf formed since the plant became diseased. The first sign of the disease is the appearance of nearly colourless spots upon the lowest exposed portions of the youngest leaves. As more of each leaf becomes exposed by growth the youngest portions exhibit a progressive increase in the frequency of the spots. Soon the spotting becomes general over the whole of the base of the leaf, and continues to appear at uniform frequency over all the new leaves subsequently formed. The virus of maize streak is not sap-transmissible, and the insect vectors are the leaf hoppers *Cicadulina* (*Balclutha*) *mbila*

Naude and *C. zeæ*. The disease is not soil- or seed-borne, and the virus has been proved by means of the special insect-feeding

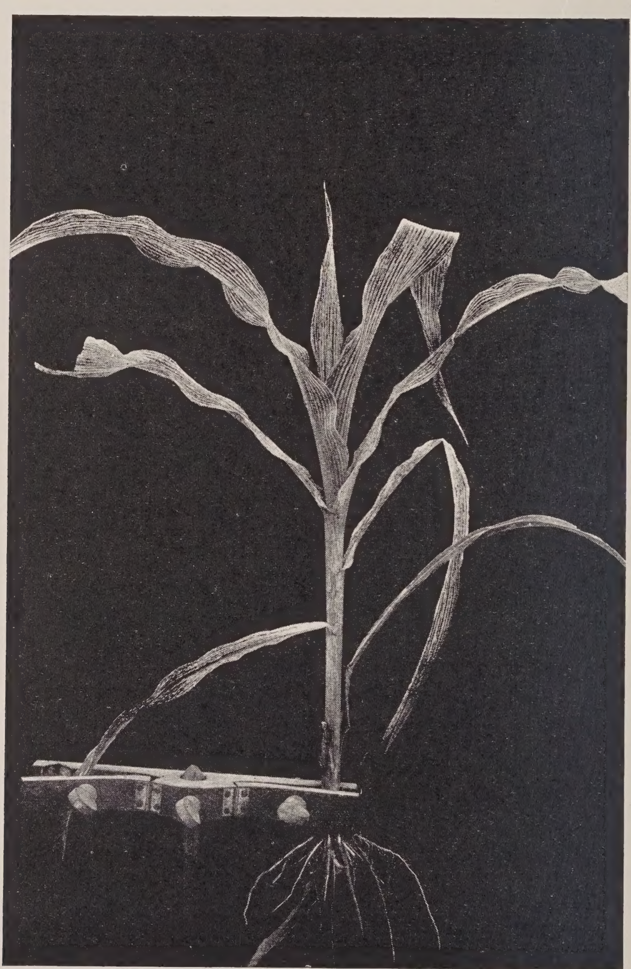

FIG. 67.—Streak disease of maize. Disease fully developed in a maize plant naturally infected in the open at an early stage of growth, showing the typical disease pattern (one-fifth natural size). (After Storey.)

technique (see Chapter V.) to pass the Berkfeld V and N filters and the L_3 Pasteur–Chamberland candle (87, 88, 90, 92).

Leaf Stripe of Maize. The symptoms of this disease resemble closely those of streak, but the insect vector is a Fulgorid, *Peregrinus maidis*, which is unable to transmit the virus of maize streak. Leaf stripe is therefore most probably due to a different virus. It occurs in Cuba and Hawaii (49, 85). The maize plant is also susceptible to the virus of sugar cane mosaic.

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GLOSSARY OF TERMS USED IN VIRUS STUDIES

Acronecrosis. Necrosis of the top of the plant; a word used by Quanjer to describe a necrotic disease of the potato in which necrosis begins at the top of the plant and progresses downwards. (See Potato Virus X, p. 309.)

Acropetal. From below upwards; a word used by Quanjer to describe a necrotic disease of the potato where necrosis begins on the lower leaves and lower part of the stem and progresses in an upward direction. (See Potato Virus Y, p. 311.)

Active, Inactive. Words used by Storey to differentiate between races of an insect species, normally a vector of a virus, which are able to transmit (active) and unable to transmit (inactive) the virus.

Agamic. Reproducing without union with a male.

Amphoteric. Descriptive of substances which can function as acids by forming salts with bases, and also as bases by forming salts with acids.

Antibody. A substance in, or a property of, blood or other body-fluids of an animal that has been injected with an antigen or infected by a microbe containing the antigen. By virtue of this substance the fluid reacts with the antigen, neutralizing, precipitating or agglutinating it. (Gardner.)

Antigen. A substance which, when injected into an animal, causes the production of a specific antibody. (Gardner.)

Basipetal. Towards the base.

Carrier (insect). A word, now obsolete and replaced by " vector," to designate an insect which transmits a virus from one host to another.

Carrier (plant). A plant which bears a concealed virus within it. Such a plant shows no symptoms of disease but can act as a source of infection to other non-carrying susceptible hosts.

Cataphoresis. An electrical method of separating out particles in a fluid, taking advantage of their electro-positive or -negative properties. (Gardner.)

Chlorosis. Blanching of the normal green colour of leaves or turning green of petals, etc.

Dendritic. Exhibiting tree-like or moss-like markings.

Elytrosoma. A name given by Schaffnit and Weber to certain spindle-shaped bodies occurring in the phloem of mosaic-infected sugar beet.

Enation. A cup-like or foliar outgrowth from the veins on the under sides of leaves, induced by certain virus affections.

Filtration End-point. A term used in filtration work with collodion membranes to indicate the average pore diameter of the limiting membrane.

Flavescent. Descriptive of leaves which exhibit abnormally light green or yellow areas.

Grafting. The insertion of a scion of one plant into the stock of another so that organic union results.

Infectious Variegation. A term usually applied to mosaic diseases of the type found in *Abutilon*, which are apparently transmissible only by grafting.

Infective. This word is generally used to indicate " virus-bearing," with particular reference to insect vectors of viruses. Such insects are capable of infecting healthy susceptible plants with the virus. It must be borne in mind, however, that Storey has shown that certain insect individuals exist, of a species normally a vector, which, while *virus-bearing*, are not *infective* in the sense of being able to convey infection, unless the wall of the alimentary canal is artificially punctured. (See p. 157.)

Inoculation. The usual English meaning of this word is the actual application, by rubbing or scratching, of virus fluid to the tissues of the plant to be infected. In America and elsewhere the word has the wider meaning of conveying infection to a host by any method of disease transmission.

Necrosis. A destruction or dying of the cells either in leaf or stem.

Net Necrosis. A necrosis of the phloem in potato tubers affected in the current season with leaf roll ; recorded only from America.

Pseudonet-Necrosis. A name given by Quanjer *et al.* to an internal parenchyma necrosis of potato tubers, occurring in the form of numerous brown spots both inside and outside the xylem ring. This condition has been re-named by Quanjer (*Phytopath.*, 1931) *phloem-parenchyma necrosis* of the potato tuber.

Ruffling. A slight crumpling of the leaf surface ; a milder form of rugosity.

Rugose. Descriptive of a condition of wrinkling or corrugation of the leaf surface, usually accompanied by mottling.

Rust. A necrosis of the foliage of potato plants in which the affected leaves present a rusty appearance. This condition occurs with several potato virus diseases and has only been observed out-of-doors. It is probably secondary to the virus infection due to particular environmental conditions not at present understood.

Savoying. An appearance, induced by virus affections in leaves, which presents a superficial resemblance to a savoy cabbage.

Scolescoma Anemones. A name given by Klebahn to oval bodies found in the living phloem cells of *Anemone nemorosa* affected with alloiophylly.

Thermal Death-Point. The temperature at which a virus becomes inactivated.

Vector. An agent, usually an insect, which transmits a virus from one host to another.

Viriferous. An American word descriptive of " virus bearing," usually with reference to a virus-bearing insect.

Virosis. An American word coined to describe a *virus disease* as distinct from a *virus*.

Viruliferous. See viriferous.

X-body. A name first given by Goldstein to the intracellular inclusions characteristic of some plant virus diseases.

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